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FLOUR QUALITY AND PROTEOLYSIS

M. KOLOSTORI

(Received September 7, 1977; revision received April 19, 1978; accepted April 27, 1978)

The protease activity in flours of various wheat varieties was studied on a synthetic substrate, N-α-benzoyl-DL-arginine-4-nitroanilid (DL-BAPA). Protease activity was found to depend largely on the wheat variety and conditions of cultivation. The correlation between various methods of protease activity measurement was studied using bacto-haemoglobin or DL-BAPA as substrate. The correlation discovered was characteristic only within variety, which points to the presence of different proteases in wheat. The effect of protease upon the physical characteristics of dough and the volume of the test loaf was studied in model experiments with papain. Hereafter the protease activity of many wheat flours was measured on bacto-haemoglobin and DL-BAPA substrates and the baking properties of the flours were established. With some of the wheat varieties protease activity significantly affected the baking properties. However, no close correlation was found between flour quality and protease activity.

The physical characteristics of doughs prepared from wheat flour are determined mostly by the gluten proteins. Changes in these proteins cause changes in the physical characteristics of doughs and the quality of the end product. By breaking the peptide bonds of the gluten proteins proteases have a softening effect on the dough.

Data found in the literature show cereal proteases to be of the papain type (SH proteinases). Their activity may be increased with cysteine, glutathione or other reducing agents (BALLS & HALE, 1938). However, some conflicting views were also encountered (HITES *et al.*, 1951).

The analysis of proteases extracted from wheat flour by starch-gel electroforesis (KAMINSKI & BUSHUK, 1969) and their fractionation by gel chromatography (WANG & GRANT, 1969) indicate that wheat flour contains several proteases.

HANFORD (1967) differentiates between alfa- and beta-proteases in wheat flour. The alfa-protease is responsible for the softening of gluten, the beta-protease for the increase in acid-soluble nitrogen. The pH optimum of beta-proteinase falls in the mildly acid region and that of the gluten softening alfa-proteinase is at pH 7–8.

In the aleurone fraction and in the endosperm BELITZ and LYNEN (1974) found two types of proteases, which differ in their pH optimum, in their behaviour to indicators and in their molecular weight.

"Enzyme pH 5" degrades haemoglobin and casein at about pH 4–5. The reaction is not inhibited by diisopropyl fluorophosphate (DFP) and iodine acetate.

"Enzyme pH 7" breaks down trypsin substrate N-alfa-benzoyl-DL-arginine-4-nitroanilid (DL-BAPA) at a pH optimum of 7.5. It does not degrade N-succinyl-L-phenylalanine-4-nitroanilid, the substrate of chymotrypsin. Its activity may be inhibited with DFP and this points to the presence of serine protease. Its similarity to trypsin appears not only in its substrate specificity but in its reaction to inhibitors as well. This enzyme or group of enzymes may be inhibited by 1-chloro-3-tozylamino-7-amino-heptanone-(2), which is a specific trypsin inhibitor. On the other hand, the chymotrypsin inhibitor 1-chloro-3-tozylamino-4-phenyl-butanone-(2) does not affect activity.

According to BELITZ and LYNEN (1974) further investigations are necessary to show whether "enzyme pH 7" is identical with the gluten-softening alfa-protease described by HANFORD (1967).

BREYER and HERTEL (1973) studied protease activity in wheat and rye flour using BAPA. The advantage of this method against those applying natural substrates lies in the use of a synthetic substrate of constant and well-defined composition. This results in a known hydrolysis product the concentration of which is measurable by colorimetry.

The protease activity of a great number of wheat varieties was determined on bacto-haemoglobin and DL-BAPA substrates in order to be able to compare the two methods and their effect on the physical characteristics of the dough and on the volume of the end product.

1. Materials and methods

The flour of about 0.5% ash content, used in the experiments was prepared on a laboratory mill.

The physical characteristics of the dough were studied with a *Farinograph*, an *Extensograph* and a *Maturograph* (POMERANZ, 1971).

The behaviour of the dough during kneading was characterized by a quality index (Q) determined on the basis of the farinogram (Hungarian Standard 6369/6–73).

For the *Extensograph* and *Maturograph* measurements the dough was prepared in the *Farinograph* (kneaded in a 300 g bronze trough for 3 min). When prepared for the *Extensograph*, 2% salt was added to the dough. The dough was allowed to stand for 45 min, then it was stretched in the *Extensograph* and the resistance to extension was measured in *Brabender* units (BU).

For studies on the *Maturograph* 2% salt, 4% yeast and 0.5% sugar were added to the dough. After kneading the dough was allowed to stand for

15 min. In the *Maturograph*, used to study the dough during raising, the proofing period and the resistance (BU) were measured at the optimum of raising.

To study the effect of protease activity upon the quality of the gluten, the gluten was washed on the *Gluto-Matic* apparatus (WALTL, 1972) and the spread (extensibility) of a gluten ball of predetermined weight was measured in mm.

To determine the relationship between protease activity and the quality of the product a baking test was carried out. A rapid laboratory mixing machine (Type *Diosna*) was used to prepare the dough (with 4% yeast, 2% salt and 0.5% sugar). The proofing period was 30 min. Three test loaves were made of each flour sample by applying proofing periods of 50, 60 and 70 min, resp., in order to establish the optimum duration. The volume of the loaf having the best shape was determined by the mustard seed displacement method.

The effect of protease activity on the physical properties and loaf volume was studied by model experiments with papain (*Merck*, No. 7144).

The protease activity was determined either according to AYRE and ANDERSON (1962) on bacto-haemoglobin substrate or according to BREYER and HERTEL (1973) on DL-BAPA synthetic substrate (*Merck*, No. 1670). With the first of the two methods the non-degraded substrate was precipitated with trichloro acetic acid and the soluble nitrogen was determined according to *Kjeldahl*. The activity was expressed in haemoglobin units per g (HU g^{-1}). Two parallel determinations were made of each sample.

With the second method the protease extract obtained from the flour was centrifuged and filtered and added to the DL-BAPA solution. The p-nitro-anilid formed by enzymatic hydrolysis was determined quantitatively by measurement of the optical density at 400 nm on a *Spektromom* 361 type spectrophotometer. Protease activity is in proportion with the change of optical density during 15 min (ΔE). Two parallel measurements were made with each flour sample.

Correlation between protease activity and the methods of flour analysis were tested by regression analysis (SVÁB, 1973).

2. Results

Protease activity is illustrated by some model experiments with papain (Fig. 1). It may be seen that as a result of papain addition the dough became softer, its resistance to extension decreased and it lowered the resistance of the dough and the proofing period as measured on the *Maturograph*.

The effect of the addition of papain on the volume of the test loaf is shown in Table 1. As can be seen, the addition of papain reduces the volume of the test loaf.

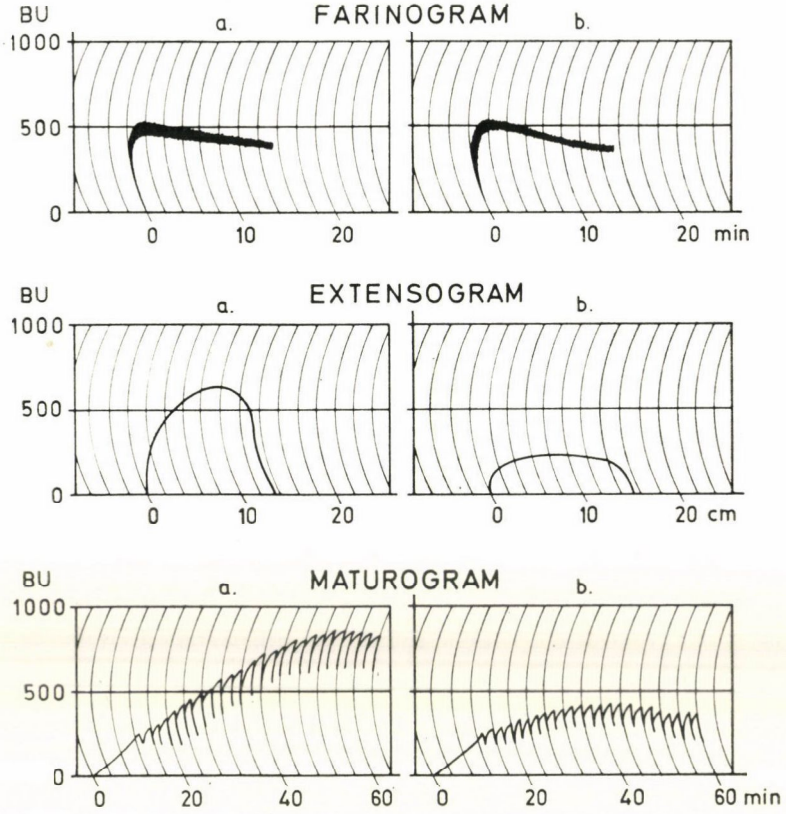

Fig. 1. The effect of papain addition on the physical properties of the dough. Papain: *Merck*, No. 7144. a: Dough without papain; b: Dough prepared with 27 ppm papain

Table 1

The effect of papain on the volume of the test loaves prepared from 6 flour samples of different quality, milled in the laboratory

Papain: *Merck*, No. 7144
For the baking test see para. 1

Serial number of sample	Volume of the control loaf (cm^3)	Volume of the loaf baked with 27 ppm papain (cm^3)
1	1300	985
2	1210	955
3	1250	850
3	1240	895
5	1210	920
6	1115	875

The protease activity is dependent on the wheat variety. This is shown in Fig. 2, where the protease activity of wheat varieties, grown under identical conditions, is illustrated. The data pertain to wheat harvested in 1974 and 1975. Variety *Kompolti* was of low protease activity in both seasons and at both-places of cultivation. The highest protease activity was shown in variety *KGI-K2*.

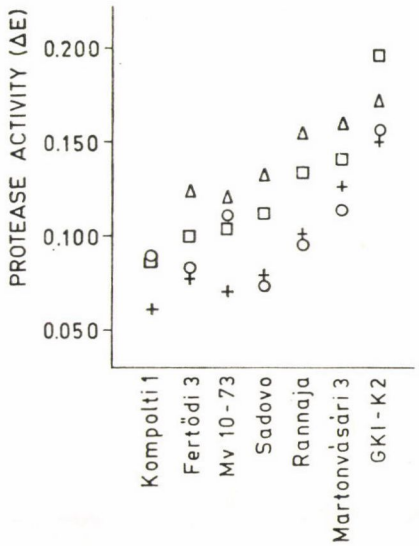

Fig. 2. Protease activity of different wheat varieties. Substrate: $5 \cdot 10^{-3}$ *M* DL-BAPA solution. Buffer: 0.1 *M* acetic acid, pH = 7.0. Stabilizer: $1.65 \cdot 10^{-3}$ *M* cysteine solution and $1.65 \cdot 10^{-3}$ *M* ethylene diamine tetraacetic acid solution. Temperature: 33 °C. Wavelength: 400 nm. Place of cultivation: + Eszterág, 1974; □ Székkutas, 1974; ○ Eszterág, 1975; △ Szombathely, 1975

The protease activity in various wheat varieties is highly affected by conditions of growing. The protease activity of 4 varieties as a function of the site of cultivation is shown in Fig. 3 (wheat harvested in 1975). The lowest activity was measured in wheat samples originating from the town of Székkutas and the highest in samples from the Szombathely region.

In order to be able to compare protease activity as measured on natural and synthetic substrates, resp., 34 flour samples were tested. No correlation was established between the protease activities of 10 wheat varieties (1–8 flour samples per variety) as measured on bacto-haemoglobin and on BAPA, resp.

However, on plotting the 34 pairs of data, a certain correlation appeared between the activities as measured by the two different methods. The correlation seems to be different for each wheat variety. Fig. 4 shows the values belonging to varieties *Martonvásári* 3, *Bezostaya* and *Kavkaz*. These are placed along a straight line, while a different correlation is valid for variety *GK Fer-*

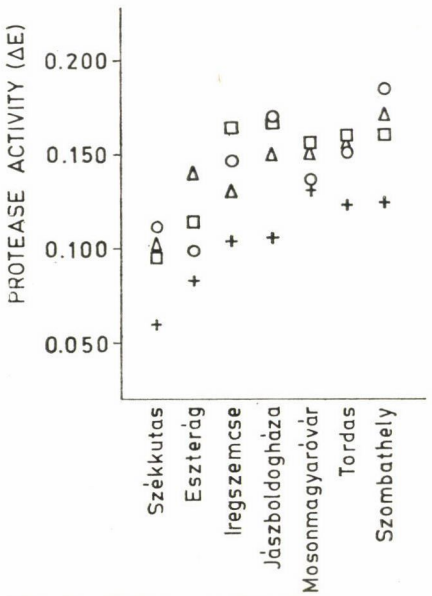

Fig. 3. Protease activity of wheat flours as a function of the place of cultivation. Substrate: $5 \cdot 10^{-3}$ *M* DL-BAPA solution. Buffer, stabilizer, temperature, wavelength as in Fig. 2. Wheat varieties: □ *Martonvásári* 3: △ *Bezostaya;* ○ *Kavkaz;* + *GK Fertődi 3*

Table 2

Relationship between protease activities as measured on bacto-haemoglobin and on BAPA, respectively

Wheat varieties tested:

Kavkaz	8 samples	*Mironovskaya* 808	2 samples
Bezostaya	6 samples	*Rannaya*	2 samples
GK Fertődi 3	5 samples	*Libellula*	1 sample
Martonvásári 3	5 samples	*Trapezica*	1 sample
Avrora	3 samples	*Burgas*	1 sample

Wheat samples were milled on a laboratory equipment

Wheat variety	Coefficients of determination		Number of data pairs
	linear	quadratic	
10 wheat varieties	0.000	0.002	34
Martonvásári 3, *Kavkaz*, *Bezostaya*	0.548***	0.597	15
GK Fertődi 3	0.948***	0.950	5

*** Significant at the 1% level of probability

tődi 3. The pertinent coefficients of determination are given in Table 2. The table shows the percentage of probability where the linear correlation is significant. Fig. 4 shows the regression lines.

The relation between the physical characteristics of the dough and the volume of the test loaf on one hand and the protease activity on the other, is summarized in Table 3. The correlation coefficients related to the quantity of gluten and spread of gluten are also given.

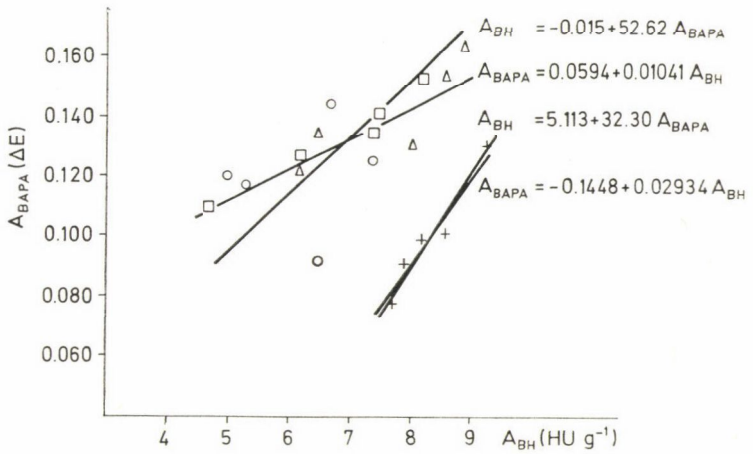

Fig. 4. Relationship between protease activities as measured on bacto-haemoglobin (A_{BH}) and BAPA substrates (A_{BAPA} = ΔE). A_{BH} substrate: Difco bacto-haemoglobin; puffer: acetate pH = 4.7; temperature: 40 °C. A_{BAPA} substrate: substrate, buffer, stabilizer, temperature, wavelength as in Fig. 2. Wheat varieties: □ *Martonvásári* 3; △ *Bezostaya;* ○ *Kavkaz;* + *GK Fertődi* 3

The role of protease activity in the development of the physical properties of the dough and the volume of the test loaf could not be proven unambiguously by two-variable regression analysis. Of the physical properties, only the negative effect on spread of gluten could be proven by mathematical statistical methods. With increasing extensibility, the quality index decreased, the fermentation period and the resistance of dough, as measured with the *Maturograph*, increased. For the volume of the test loaf the quantity of gluten is decisive. Increasing the amount of gluten resulted in an increase in the volume of the test loaf.

The correlation between the physical properties, the volume of the test loaf and protease activity was studied in each of four wheat varieties. In the case of varieties *Martonvásári* 3, *GK Fertődi* 3 and *Bezostaya* the correlation was significant at the 1–10% level (Table 4), when measured with bacto-haemoglobin.

Table 3

Relationship between the physical properties of the dough the volume of the test loaf, the quantity and spread of gluten and the protease activity in the flour as expressed by correlation coefficients

	Quantity of gluten, %	Spread of gluten, mm	Protease activity (DL-BAPA) ΔE	Number of data pairs
Quality index	0.059	−0.557****	0.288***	176
Resistance to extension (BU)	−0.262**	−0.569****	0.084	90
Resistance by *Maturograph* (BU)	0.178	0.605****	0.251	36
Proofing period (min)	−0.200	0.483***	0.127	36
Loaf volume (cm^3)	0.449****	0.039	0.341****	176

	Quantity of gluten, %	Spread of gluten, mm	Protease activity (bacto-haemoglobin), HU g^{-1}	Number of data pairs
Quality index	0.209*	−0.663****	0.095	76
Resistance to extension (BU)	−0.174	−0.634****	−0.001	33
Resistance by *Maturograph* (BU)	0.119	0.474**	−0.412**	29
Proofing period (min)	−0.129	0.408**	−0.152	29
Loaf volume (cm^3)	0.304***	−0.119	−0.268**	76

* Significant at the 10% level of probability
** Significant at the 5% level of probability
*** Significant at the 1% level of probability
**** Significant at the 0.1% level of probability

Table 4

Relationships between the physical properties of the dough, and the volume of the test loaf on the one hand and protease activity (on bacto-haemoglobin), on the other, in wheat varieties Martonvásári 3, GK Fertődi 3 *and* Bezostaya

	Coefficients of determination		Number of data pairs
	linear	quadratic	
Quality index	0.147***	0.156	45
Resistance to extension (BU)	0.264*	0.508	14
Resistance by *Maturograph* (BU)	0.070	0.077	24
Proofing period (min)	0.000	0.042	24
Loaf volume (cm^3)	0.091*	0.107	45

* Significant at the 10% level of probability
*** Significant at the 1% level of probability

Table 5

Relationship between the physical properties of the dough, and the volume of the test loaf on the one hand and protease activity (on DL-BAPA), on the other, in wheat variety GK Fertődi 3

	Coefficients of determination		Number of data pairs
	linear	quadratic	
Quality index	0.357***	0.369	18
Resistance to stretching (BU)	0.619**	0.741	7
Resistance by *Maturograph* (BU)	0.105	0.474	8
Proofing period (min)	0.072	0.079	8
Volume of loaf (cm^3)	0.127	0.204	18

** Significant at the 5% level of probability
*** Significant at the 1% level of probability

As far as BAPA-ase activity is concerned, the variety *GK Fertődi* 3 differed somewhat from varieties *Martonvásári* 3 and *Bezostaya*. At identical physical dough properties the BAPA-activity of *GK Fertődi* 3 was lower than that of the other two varieties. The physical characteristics of variety *GK Fertődi* 3 and its BAPA-ase activity showed a significant correlation at the 1–5% level of probability (Table 5).

In varieties *Martonvásári* 3 and *Bezostaya* no correlation was found between the physical properties and the loaf volume on the one hand and the BAPA-ase activity on the other.

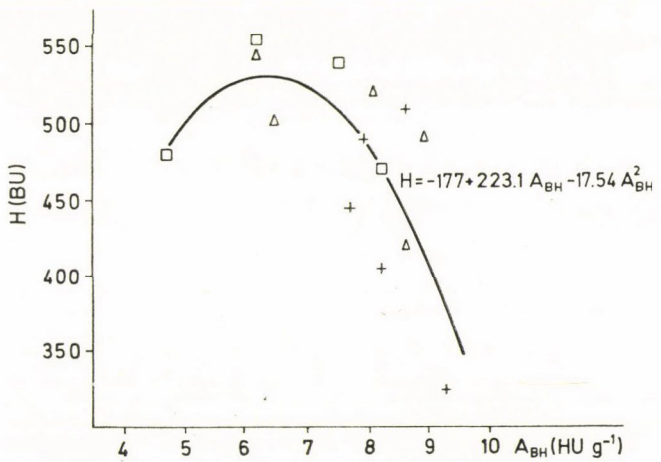

Fig. 5. Relationship between the protease activity (A_{BH}) and the resistance to extension (H) in different flours. A_{BH} substrate: DIFCO bacto-haemoglobin. Buffer: acetate pH = 4.7. Temperature: 40 °C. H: extension of the dough in the *Extensograph*. Fermentation period: 45 min. Wheat varieties: □ *Martonvásári* 3; △ *Bezostaya;* ○ *Kavkaz;* + *GK Fertődi* 3

The physical properties and the loaf volume showed no protease dependence with variety *Kavkaz*. The lower quality characteristics of variety *Kavkaz* may not be traced back to protease activity since at the same protease activity level varieties *Martonvásári* 3, *Bezostaya* and *Fertődi* 3 gave higher quality indices and a higher resistance to extension.

The results for which the correlation was close or rather close are illustrated in Figs. 5 to 7. The figures represent the regression equations.

No significant correlation was found between protease activity and gluten spread. The spread of gluten characterizes its quality. In the formation of gluten properties the proportions between fractions (the proportion of

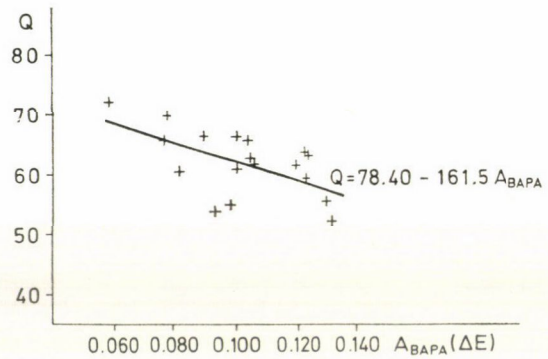

Fig. 6. Relationship between the protease activity (A_{BAPA}) and the quality index (Q) of *GK Fertődi* 3 flour. A_{BAPA} substrate: DL-BAPA; buffer: acetate pH = 7.0; stabilizer: cysteine–ethylene diamine tetraacetic acid; temperature: 33 °C; wavelength: 400 nm; Q: determined by *Farinograph*. Wheat variety: + *GK Fertődi* 3

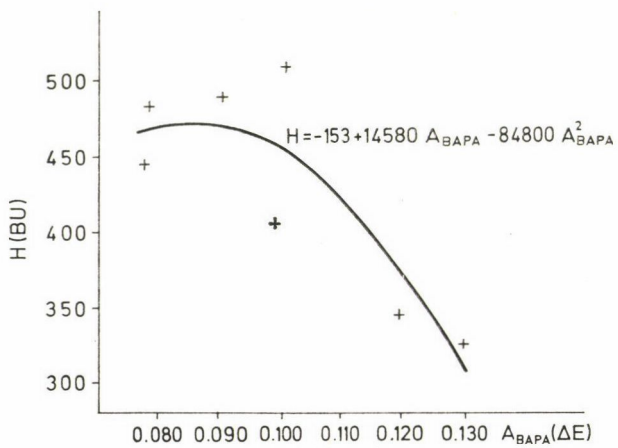

Fig. 7. Relationship between the protease activity (A_{BAPA}) and the resistance to extension of the dough (H) in the flour of *GK Fertődi* 3 wheat variety. A_{BAPA} substrate: DL-BAPA; buffer: acetate pH = 7.0; stabilizer: cysteine–ethylene diamine tetraacetic acid; temperature: 33 °C; wavelength: 400 nm; H: extension of the dough by Extensograph; fermentation period: 45 min. Wheat variety: + *GK Fertődi* 3

small and large protein molecules) and the relations and interactions between the fractions (disulfide bonds and H-bonds) are of importance (LÁSZTITY, 1975).

In contrast to the experience gained in model experiments with papain, the effect of protease activity could not be proven in *Maturograph* tests. This is probably the consequence of the fact that during fermentation the processes become more complex in the dough and the loosening of the dough comes in the foreground.

3. Conclusions

Between protease activities as measured with DL-BAPA and bacto-haemoglobin, resp., close correlation was observed only within variety. Data in the literature indicate the presence of two different proteases in wheat flour, differing in their pH optimum. While the pH optimum of protease I lies in the mildly acid range that of protease II is about pH 7–8. On the basis of the assays of BELITZ and LYNEN (1974) it may be presumed that with bacto-haemoglobin protease I, measured at a pH optimum of 4.7, and with DL-BAPA protease II measured at a pH optimum of 7, these are responsible for the loose correlation between the two methods.

No unambiguous correlation was found between protease activity on the one hand and the physical properties of the dough and the volume of the loaf, on the other. In the physical properties of the dough and the volume of the loaf, other flour characteristics (the quantity and the quality of gluten) play an important part.

Out of the physical properties of dough, only the quality index and the resistance to extension showed a significant correlation with protease activity, if data were grouped according to variety. However, correlations were not close.

Sound flour samples of about 0.5% ash content were used in the tests. Protease activity is not evenly distributed in the wheat kernel. The highest protease activity was found in the aleurone layer and in the germ. Thus, the protease activity of flours of higher ash content is higher. This permits of extending the measuring range of protease activity and, thereby, improving the correlations.

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CHARACTERISTICS OF THE MICROBIOLOGICAL STATE OF TOBACCO PRODUCTS

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In order to evaluate the microbiological state of Hungarian tobacco products, the whole range of these products was investigated. The differences found between products could be traced back to the quality of the tobacco used, the technology applied and the physical chemical characteristics of the product.

The microbial contamination of the various tobacco products: cigarettes, cigars and cigarette and pipe tobacco by mesophilic aerobic cells was 10^5, 10^6 and 10^5 g^{-1}, resp., with yeast cells at 10^2, 10^3 and 10^{2-3} g^{-1}, resp., with mould cells at 10^2, 10^3 and 10^4 g^{-1}, resp. Infection at this level did not cause any change in the sensory quality of the products.

The majority of mould strains isolated and identified in cigarettes were characteristic members of the flora of the storage room: *Penicillium* and *Aspergillus* strains. However, representatives of the field flora were also isolated, such as various species of *Alternaria, Cladosporium* and *Fusarium.*

Considering the species identified, theoretically the occurrence of mycotoxins in tobacco cannot be excluded.

The microbial flora of tobacco products was studied only by a few authors. This is due to the fact that tobacco products are rarely prone to microbiological spoilage. This occurs only where the relative humidity of air is above 75% and the moisture content of the cut tobacco exceeds 65–70%.

A study of the related literature shows that research in the field of tobacco has been mainly concerned with the microbiological aspects of phytopathology (GULYÁS, 1965; KIRÁLY, 1968) and preparation of tobacco (GULYÁS, 1958; GARAGULY & PÓLYA, 1958; GIOVANOZZI, 1961; GARAGULY, 1963, 1964; SCHMIDT, 1964; WELTY *et al.*, 1968; WELTY & LUCAS, 1968a,b; WELTY & STOUT, 1975; WELTY & WEEKS, 1975) as well as storage of the basic material (YANG & LUCAS, 1970; LUCAS *et al.*, 1973). Data related to the final product were not found.

In view of the fact that these products are burned in their proper use, the purpose of the present investigations may be questioned. The temperature of the glowing tip is 823–1073 K (550–800 °C). At these temperatures all microorganisms and the toxic products of metabolism are destroyed by burning.

While planning our investigations we set out from the following considerations:

– since tobacco products are consumer goods, under exceptional conditions they may act as sources of contamination,

– due to the chemical composition of tobacco it is a suitable nutrient medium for the growth of microorganisms. These may disadvantageously affect the sensory quality of tobacco, its quality and, as a function of the ecological parameters, may form a limiting factor in the keeping quality of tobacco products,

– the presence of moulds always carries the danger of mycotoxins and their inhalation at the temperature of the main stream of smoke [293–298 K (20–25 °C)] is not excluded,

– the microbiological condition of the final product permits of drawing conclusions as to the sanitary and technological level of preparation and processing.

On the basis of the above we set the following aims:

– establishing and comparing the microbiological level of Hungarian and foreign tobacco products,

– determination of the representative mould strains isolated from cigarettes.

In the course of fermentation of tobacco GIOVANOZZI (1961) found mesophilic cell counts of 2×10^6 and during the most intensive phase 5×10^8–10^9 g^{-1}. In the initial phase of fermentation the *Blastomycetes*, later the *Schizomycetes* predominated. In the fermented tobacco cocci, and members of the genus *Bacillus* prevail.

GULYÁS (1965) found the following yeast strains in fermenting tobacco: *Hansenula anomala*, *Candida guillermondii*, *Torulopsis dattila*, *Rhodotorula mucilaginosa*.

WELTY and STOUT (1975) describe data on the quality and quantity of fungi surviving preparation.

NIKODÉMUSZ (1972) discovered microbial spoilage in Cuban cigarettes. The white spotted cigarettes of pungent smell and taste contained 1.5×10^6 *Bacillus subtilis* cells per g and 1×10^5 mould cells per g.

The technological phases of preparation of tobacco for processing do not destroy completely the microorganisms, but change the composition of the microflora. Thus it may be seen that tobacco and tobacco products are always contaminated with microorganisms.

1. Materials and methods

The County Institute for Food Control and Chemical Analysis, Debrecen is charged with studying the activities of the tobacco industry, with the control of the quality, as set in the National Standards, and microbial load of its products.

1.1. Materials

In the Debrecen Tobacco Factory and in the distribution centers of the counties Szabolcs-Szatmár and Hajdú-Bihar checking combined with sample taking was regular. However, in the factories in Eger, Pécs and Sátoraljaújhely and in the retail network checking was occasional.

During 1974–1975 two-element samples were analysed of the 33 kinds of Hungarian cigarettes on 288 occasions, of the 9 cigar varieties on 32 occasions and of the 10 cigarette and pipe tobacco varieties 25 times.

For the sake of comparison the two-element samples of 33 foreign cigarettes, of 5 kinds of cigars and of 8 kinds of cigarette and pipe tobacco were examined for their microbial contamination.

1.2. Methods

1.2.1. Microbiological methods. To establish the microbial contamination in tobacco products culturing, microscopic and humid chamber methods were applied.

1.2.1.1. Determination of the total mesophilic aerobic viable cell count – Ten g of the homogenized cut tobacco (with cigars including the cover leaves and envelope) were weighed (on *OWA* rapid balance to mg accuracy) and suspended in 90 ml of sterile pepton water to obtain a stock suspension. This was resuscitated for 45 s and homogenized for 2.5 s at 20 000 rpm (Type *UNIPAN*-309 Polish blade-homogenizer). After a sedimentation of 15 s, the pH was measured.

Decimal dilutions were prepared with sterile pepton water (0.1% pepton, 0.01% Tween-80), using a test-tube homogenizer, to obtain dilutions of 10^{-6}–10^{-9}, as required.

The viable cell count was determined by the Most Probable Number (MPN) method in tryptone-glucose-yeast extract nutrient. The dilutions were incubated at 303 K (30 °C) for 48 h in a laboratory thermostat (Type LP-104, Hungarian product). *Hoskins'* Tables were used to evaluate the results. The latter were expressed in cell per g as the average of two measurements.

1.2.1.2. Determination of yeasts – From the stock suspensions described above two 0.1-ml portions were inoculated in malt agar of pH 4.5 (acidified with citric acid) and incubated at 298 K (25 °C) for 48–72 h.

In order to inhibit the growth of bacteria prior to distribution the nutrient agar was cooled to 318 K (45 °C) and 100 g ml^{-1} chlortetracycline were added.

When evaluating, dishes containing 20–300 colonies were taken into account. Averages were calculated subsequent to logarithmic transformation of the parallels.

1.2.1.3. Determination of the propagule count – From dilution steps 10 to 10^5 0.5 ml were inoculated into previously desiccated *Czapek–Dox* and malt nutrients containing antibiotics, forming 2 parallels. These were incubated at 298 K (25 °C) for 4–7 days. Dishes containing 5–50 colonies were evaluated. Results are given as above.

Based on the experiments of WELTY and LUCAS (1968), the saline malt agar and the saline *Czapek–Dox* agar (with 10% added NaCl) were tested. In addition to the mould colonies the development of yeasts and micrococci was observed. Due to their halophilic character and resistance to drying the proliferation of *Debaryomyces* yeasts and of cocci is possible, since these may be isolated from the dry tobacco, too.

The *Rosebengal*–streptomycin sulfate-malt agar was successfuly used. This nutrient was complemented with 0.5 g KH_2PO_4, 0.5 g K_2HPO_4, 0.5 g $MgSO_4$ $7H_2O$, 0.05 g *Rosebengal* and 0.03 g streptomycin sulfate. The members of the *Aspergillus glaucus* group and *Alternaria* species grow well on this nutrient and the latter impedes the growth of a number of rapidly developing fungi.

The nutrients for the cultivation experiments were composed of Oxoid and Difco preparations.

1.2.2. Determination of moulds isolated from cigarettes. From the nutrients as described at the determination of the propagule number 182 strains were isolated. Their pure culture was developed on *Czapek–Dox* nutrient.

To isolate the members of the genus *Aspergillus* a selective nutrient was used. The Aspergillus Differenciating Medium (ADM) serves mainly for the cultivation of *Aspergillus flavus*.

In identifying the fungi thus cultured the works of UBRIZSY and VÖRÖS (1968) and VÖRÖS and UBRIZSY (1960) were used. To identify *Aspergilli* the taxonomic key of RAPER and FENNEL (1965) and for *Penicillia* that of RAPER and THOM (1949) was used.

Two moulds were determined at the Botany Department, Kossuth Lajos University, Debrecen.

1.2.3. Physico-chemical analysis. The microbiological tests were complemented by the following analyses:

– moisture content [according to Hungarian Standard 20510–74; sample size: 4–5 g, drying at 368 K (95±1 °C) for 3 h],

– equilibrium relative humidity (ERH %) according to the crystal liquefaction method of VAS and CSONTOS (1956) at 298 K (25 °C) for 24 h,

– pH of the stock solution (Precision pH meter, product of Radelkis, Budapest).

2. Results

2.1. Analyses of the products of the tobacco industry

The microbial contamination of Hungarian and imported tobacco products are shown in Figs. 1–3.

The contamination related to the group of product seems to be fairly constant. In the humid chamber, in agreement with the findings of MOLDEN-

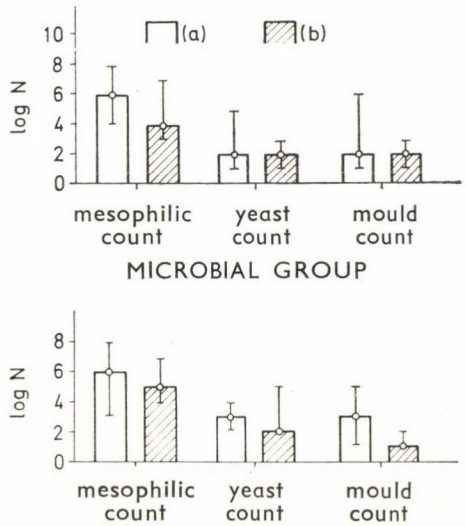

Fig. 1. Comparison of the occurrence of microbial groups in Hungarian and foreign cigarettes (N = propagules per g) (a) Hungarian (b) foreign

Maximum–minimum; ○ average of log N values

Fig. 2. Comparison of the occurrence of microbial groups in Hungarian and foreign cigars (N = propagules per g) (a) Hungarian (b) foreign

Maximum–minimum; ○ average of log N values; average value falls near the minimum

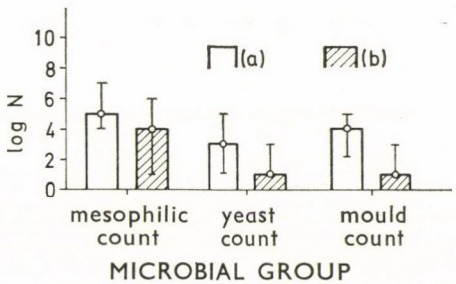

Fig. 3. Comparison of the occurrence of microbial groups in Hungarian and foreign cigarette and pipe tobacco (N = propagules per g) (a) Hungarian (b) foreign

Maximum–minimum; ○ average of log N values; average value falls near the minimum

HAUER and BERGER (1970), during a shorter or longer period (4–30 days), every product became mouldy.

The frequency distribution of microbial strains characteristic of the product is shown in Figs. 4 to 9.

Some additional physical chemical characteristics for Hungarian products are given in Table 1.

Table 1

Physical and chemical characteristics of Hungarian tobacco products

Product	Moisture content, %			Stock solution, pH	ERH, %
	Min.	Max.	Average		
Cigarettes	10.1	13.2	11.6	5.5–6.8	55.6–73.8
Cigars	9.6	12.5	10.5	5.8–7.0	55.6–63.8
Cigarette and pipe tobacco	13.0	14.0	13.5	5.5–6.5	70.3–76.0

Moisture content expressed as % calculated for wet sample, 4–5 g dried at 368 K (95 °C) for 3 h.

pH: 10 g sample homogenized in 90 ml water, allowed to sediment for 60 s, measured on a *Precision* pH meter.

ERH % determined according to VAS and CSONTOS (1956) by the crystal liquefaction method, conditioned at 298 K (25 °C) for 24 h.

2.2. *Mould strains separated and identified from cigarettes*

Since the microbial spoilage of tobacco is mainly due to damage caused by moulds, it seemed desirable to identify the strains separated. From Hungarian products marked by a quality index *(Fecske, Kossuth, Munkás, Symphonia, Románc)* 182 strains were separated. Their distribution according to genus is given in Table 2.

Table 2

Distribution according to genus of mould strains identified from cigarettes with quality index

Serial number	Genus	Number of strains	Strains %
1	*Penicillium*	75	41.2
2	*Aspergillus*	57	31.2
3	*Alternaria*	13	7.7
4	*Cladosporium*	11	6.0
5	*Rhizopus*	8	4.1
6	Others*	18	9.8

* *Trichotecium roseum, Mycelia sterilia, Spicaria violacea, Helminthosporium sp., Fusarium sp., Mucor sp.*

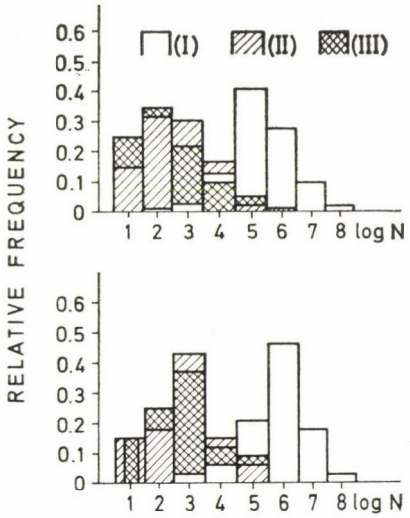

Fig. 4. Frequency distribution of microbial groups in Hungarian cigarettes (I) mesophilic aerobic viable cell count; (II) yeast count; (III) mould count

Fig. 5. Frequency distribution of microbial groups in Hungarian cigars. (I) mesophilic aerobic viable cell count; (II) yeast count; (III) mould count

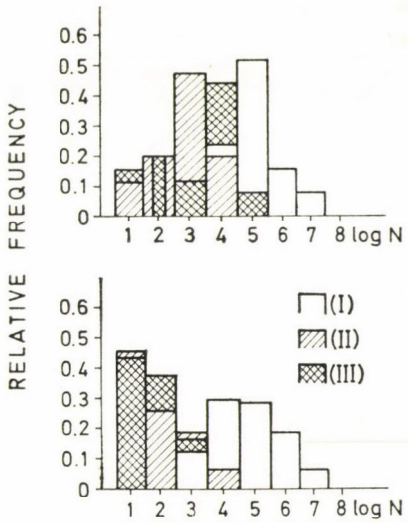

Fig. 6. Frequency distribution of microbial groups in Hungarian cigarette and pipe tobacco.
(I) mesophilic aerobic viable cell count; (II) yeast count; (III) mould count

Fig. 7. Frequency distribution of microbial groups in imported cigarettes. (I) mesophilic aerobic viable cell count; (II) yeast count; (III) mould count

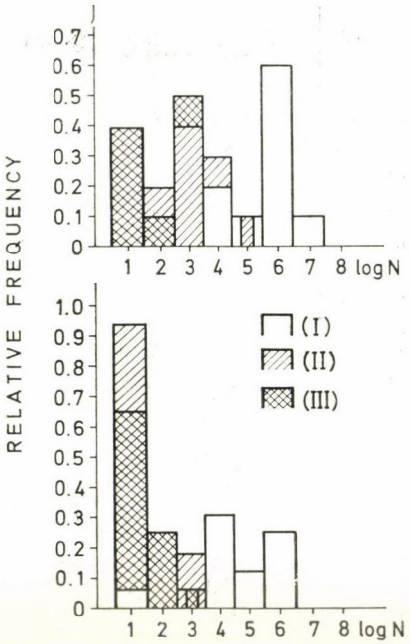

Fig. 8. Frequency distribution of microbial groups in imported cigars.
(I) mesophilic aerobic viable cell count; (II) yeast count; (III) mould count
Fig. 9. Frequency distribution of microbial groups in imported cigarette and pipe tobacco.
(I) mesophilic aerobic viable cell count; (II) yeast count; (III) mould count

From the genus *Penicillium* 17 strains were identified as belonging to the following species: *P. martensii* 13.3%, *P. aurantio-candidum* 10.6%, *P. expansum* and *P. frequentans* 8–8%, resp. The species rather rarely encountered were *P. meleagrinum*, *P. canescens*, *P. paxilli*, *P. velutinum*, *P. ochraceum*, *P. lanosum*, *P. lanoso coeruleum*, *P. tardum*, *P. corylophilum*, *P. steckii*, *P. stoloniferum*, *P. brevicompactum* and *P. brevicaule*.

Of the genus *Aspergillus* nine strains were identified. Fifty six per cent were found to be *Asp. flavus*, 12.2% *Asp. niger* and 10.5% *Asp. fumigatus*. The following species were identified: *Asp. ruber*, *Asp. cervinus*, *Asp. spinulosus*, *Asp. terreus*, *Asp. amstelodamii*, *Asp. repens*.

3. Conclusions

3.1. Microbiological state of the products of the tobacco industry

As can be seen in Figs. 1 to 3 the total mesophilic aerobic cell count in Hungarian cigarettes, cigars and cigarette and pipe tobacco was found to be 10^6, 10^6 and 10^5 g^{-1}, respectively. The yeast cell count was 10^2, 10^3 and

10^3 g^{-1} and the mould count 10^2, 10^3 and 10^4 g^{-1}, respectively. Simultaneously with the microbiological tests, the samples were tested organoleptically. The sensory value was found to be independent of the microbial count.

Striking are the differences between product groups. These can be traced back to the following reasons:

- different production technologies,
- raw materials and additives of different quality,
- difference in the proportion of stem cut and leaf cut.

Higher microbial contamination is definitely a consequence of the early life of the raw material. Cigar tobacco is dried under natural conditions (in the shed) and is fermented in stacks. Under these conditions microorganisms play a much greater role than under artificial conditions (redry process). Of the three product groups the mould content was highest in the cigarette and pipe tobacco, which at the same time had the highest moisture content (Table 1). Most properties may be traced back to the proportion of stem and leaf cut and to the differences in their equilibrium water content. In some of these products (mixed, garden pipe tobacco) the stem cut may amount to 30–36%. The study of KRAJCSOVICS and PÁLYI (1960) has shown that in an atmosphere of 45% relative humidity the equilibrium water content of stem and leaf cuts is nearly identical, whereas in atmospheres of 75–80% RH the equilibrium water content of the cut stem is higher by about 4–5%. While the average moisture content amounts to 13.5%, some pipe tobaccos may contain particles of 15–17% water and under non-conditioned storage conditions in rainy weather this may even increase. These centres of high moisture content permit the growth of xerophilic fungi.

TEICHMAN and GARAGULY (1967) found that the contamination was highest in the stem. The mould count of 10^3–10^4 g^{-1} is accounted for by the above.

3.2. Identified mould strains and their significance

As can be seen in Table 2, 90% of the identified mould strains in cigarettes belong to five genera and out of these *Penicillium* and *Aspergillus* represent 72.4%.

The relative frequency of the presence of *Asp. fumigatus* (in *Symphonia* and *Munkás* cigarettes) is of importance because this strain may cause lung mycosis. However, it has not been proven that this illness may be brought about by cigarettes (the spores may be inhaled from products without filter). Nonetheless, its presence deserves attention from the point of view of hygiene.

The persistence of the field flora is proven by the fact that *Alternaria*, *Cladosporium*, *Rhizopus* and *Fusarium* species may be identified from products and tobacco disinfected on the surface. However, in the products genera

belonging to the storage flora, such as *Penicillum* and *Aspergillus* dominate by a high number of species and individuals.

On the basis of the identified mould strains the presence of mycotoxins has to be reckoned with *e.g.* aflatoxin, citrinin, alternaria toxin, patulin, penicillic acid, kojic acid, sterygmatocystin, etc.

The problem arises whether a specific flora characteristic of a certain product may develop. Studies carried out hitherto seem to prove the development of a microbial flora characteristic of a group of products or a place of cultivation. The development of a predominating storage flora is dependent on the prevailing ecological factors.

3.3. Methodological notes

Cultivation tests show the presence of viable cells and thereby the microbiological state of the tobacco and the consequent potential danger. However, the character of the microbial processes occurring in tobacco is not indicated. Therefore, it seems desirable to carry out microscopic tests which help to detect the defective tobacco where, as a result of fungicidal or heat treatment fungi are destroyed.

Another fact to be taken into account is that species present only in low numbers may escape detection in the course of dilution.

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CONTRIBUTION TO THE THEORY AND PRACTICE OF THE ACIDIFICATION OF FEED AND FOOD

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A model experiment of cold fermentation was carried out using not easily acidifiable alfalfa as test material. In the course of this, the pH values of alfalfa of both low and high buffer capacity with identical sugar/buffer capacity quotients were adjusted to different acidity levels, with an inorganic acid mixture. During fermentation the effect of above factor on the initial growth rate of the microflora, the quantitative and qualitative components of microflora in the principal and secondary stages of fermentation, the total loss of solids volatilized with fermentation gases and the loss of protein occurring in acidified forages, were investigated. The amount of clostridial spores causing product spoilage was also determined.

Our results are summarized as follows:

- The so-called "useful microflora" of Hungarian alfalfa silages is essentially similar to those described in the literature, while the so-called "associated microflora", differs in respect of a few species. On the other hand, the microflora of pickled food products showed high similarity to the microflora of acidified feeds.
- At an initial pH of 4.0 associated flora will not multiply in silages. If the quotient of the sugar/buffer capacity is favourable, the losses arising in silages and the subsequent stability are limited by the ratio between the propagation rates of useful and associated microflora at the beginning of fermentation. However, this ratio can be influenced most intensively by means of the initial pH value.
- In the case of identical quotients of sugar/buffer capacity, considerably lower losses will arise in the acidified product having a sugar/buffer capacity quotient of lower numerical value if expressed in absolute value.
- In case of silages of high initial pH value, significant amounts of ammonia and volatile amines will evaporate with the fermentation gases thus, the protein losses as determined by the methods used at present, are not reliable.
- The low clostridial spore count often associated with high butyric acid content suggests that, after a precursory growth phase, the saccharolytic *Clostridia* are present in vegetative form.

Preservation by acidification is significant both in the food industry and in foraging. The principle of the process is known: the fermentable sugar content in food or forage is converted by microbial action into organic acids and the acids arisen in this way decrease the pH of food or forage at the rate of their integrated buffer capacity. In the optimal case, the cell count diminishes through the joint effect of pH value developing at a given water activity level and of the undissociated acid concentration, and the product becomes stabilized. It may be derived from the above that the measure of acidification of the basic material is characterized by the fermentable sugar content (%) and by the amount of lactic acid (%) required to achieve the final pH ensuring

stability with the initial pH of the basic material as the starting point *i.e.* by the sugar/buffer capacity coefficient (S/B_c: see section 1.3.). The final pH providing stability depends on the water activity solids content (WIERINGA, 1969). In general, however, it can be ascertained that, in case of unwilted crops, a final pH value of 4 is needed to ensure stability. Assuming, *e.g.*, the S/B_c value of the forage to be 1, this means, according to our formulation, that the fermentable sugar content of the crops in per cent w is equal to the lactic acid content required to reach the stable final pH. Consequently, the product would be stable only if all the fermentable sugar could be converted into lactic acid or the sugar not directly fermentable could become mobilized by enzymes. The first condition may not be realized owing to a number of obvious reasons.

In addition to the potential acidifiability (S/B_c), the crop to be fermented is converted into an edible product or it becomes inedible as the result of various effects of two enzyme systems. The distribution of the principal biochemical processes between the original enzyme system of the plant and the enzyme system of the multiplying microflora is shown in Fig. 1.

As it appears from the figure, the process results in the stabilization of forage. Thus the only desirable process, namely the formation of organic acids and aromatic substances and the subsequent conversion of organic acids, being the keystep in spoilage phenomena, depends on microbial enzyme activity. According to the investigations of GOUET and FATIANOFF (1964), the plant enzyme systems remain active only for some days after ensilage of the forage. The major share of enzymic processes demonstrated in Fig. 1 may result in total or partial spoilage, or in severe decrease of the internal value of silage. Thus, the actual quantity or rather the utility value of mass forages decreases, thereby reducing the produced gross amount of milk and causing the composition of milk to suffer changes. The spoilage phenomena in food, such as breakdown of pectin, defects of consistency, butyric acid flavour, flat-sour flavour (a fully homofermentative fermentation), *etc.*, are to be traced back to these enzymic processes.

For the not easily acidifiable forage plants (low S/B_c), the following methods are suggested:

- An effort should be made to retain the forage plant in its natural state (AIV method) by means of the addition of a large amount of inorganic acid (VIRTANEN, 1933; HILTUNEN, 1971).
- Retarded fermentation should be employed by reducing water activity (prewilting), though here it is to be noted that, in this case, a significant part of the fermentable sugar content is lost by respiration while the buffer capacity remains virtually unchanged. Thus, this procedure will result in safe storage only if wilting is continued to a level where practically every microbial activity is inhibited.

– Application of rapid lactic acid fermentation in order to promote propagation and activity of lactic acid bacteria.

The most important aim of these methods is to increase the ratio of sugar/buffer capacity. This may be achieved by increasing the sugar content, *e.g.* by adding molasses or cereals simultaneously with amylase enzyme, *etc.* or by

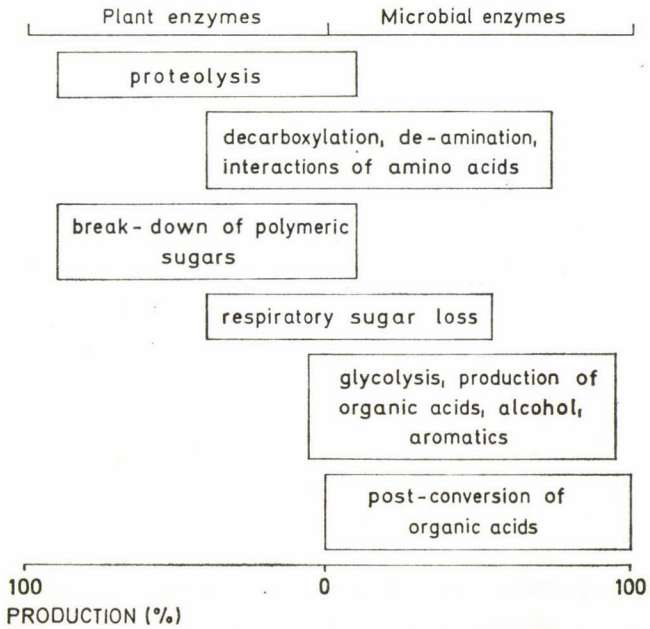

Fig. 1. The approximate distribution of the principal biochemical processes between the two main enzyme systems functioning during forage fermentation

decreasing the integrated buffer capacity (by adding acid), and thereby reducing the initial pH value thus preventing propagation of numerous proteolytic microorganisms. It is to be emphasized that the pathogen-inhibiting effect of preparations containing salts of organic acids is questionable at the given concentrations (BECK, 1968) while they form, at pH 5, a buffer system difficult to break through with lactic acid and thereby, delay the development of the final pH. Thus, the latter preparations cannot be considered additives accelerating fermentation towards stability. To promote the dominance of lactic acid bacteria it was attempted to increase their proportion by incorporating pure cultures (WIERINGA & BECK, 1964, a, b). According to other authors, the initial microflora hardly affects the microflora of the main fermentation period (WEISE, 1969). As regards antibiotics their effect is distinct, while their consequences affect unfavourably costs and animal sanitation.

The pH curves characteristic of the above-mentioned types of preservation, are demonstrated in Fig. 2.

In the course of our experiments, the qualitative and quantitative analyses of the aerobic and anaerobic microflora of alfalfa silage preserved by the AIV procedure, and the check-up of some chemical parameters characteristic of storage loss were carried out as functions of the fermentation period.

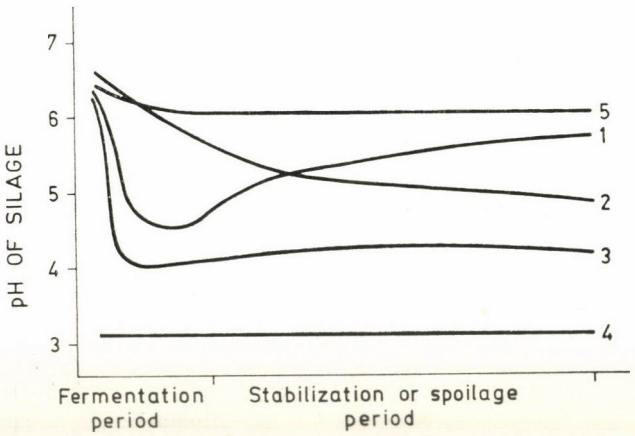

Fig. 2. The change of pH in various silages as a function of storage time (SZIGETI, 1976). 1 = forage with low S/B_c coefficient, without additive; 2 = forage preserved by increasing the dry matter content (decreasing water activity); 3 = forage preserved by an additive accelerating fermentation; 4 = forage stabilized with large doses of inorganic acids; 5 = forage preserved with a combination of antibiotics

Our aim was to examine the amount and composition of the useful and associated flora in alfalfa ensiled with different amounts of inorganic acid in model silos and the losses occurring under their influence, furthermore to investigate the clostridial spore count during fermentation.

This latter group of bacteria has an influence on cheese making, since *Clostridia* spores are not destroyed during the pasteurization of milk and they may cause butyric acid bloat in cheese. Another aim was to find an initial pH value at which the fermentative activity is reduced while the enzyme system of the plant is restrained and undesirable microorganisms will not multiply.

1. Materials and methods

1.1. Preparation of model silages

Alfalfa of the first (I) and of the second (II) cut were used as test material for examinations. The principal parameters of the test materials are shown in Table 1.

Table 1

The principal characteristics of ensiled lucerne

Lucerne	pH	S (Fermentable sugar, %)	B_c (% lactic acid)	S/B_c coeff.	Dry matter (%)	Water activity	1 kg forage contains				Ash
							crude protein	crude fat	crude fibre	nitrogen-free extract	
I	6.0	1.2	2.7	0.44	18.8	0.988	37.5	6.20	41.2	92.3	11.6
II	6.6	1.8	4.3	0.42	26.7	0.980	67.6	8.10	44.8	124.8	21.7

I = lucerne of first cut
II = lucerne of second cut

The two lucerne samples, harvested in a single course each were blended with different volumes of the seven-fold dilution of a 1 : 1 mixture of hydrochloric acid (36.5%) and sulphuric acid (98%) and placed into 5–l glass jars. After compacting, the model silos were closed on a canning factory closing machine and they were incubated at 22–24 °C. Table 2 contains the initial and final pH values developed as the effect of different treatments, and the S/B_c coefficients.

Table 2

Initial and final pH values, sugar/buffer capacity coefficients as affected by addition of the inorganic acid

Symbol of treatment*				C	1	2	3	4	5
Acid equivalent in 100 kg of green lucerne				0	5.5	11	16.5	22	27.5
Lucerne	I.	initial	pH	6.0	5.3	4.2	3.8	3.3	3.1
		final		4.5	4.2	3.9	3.7	3.3	3.2
		S/B_c		0.44	0.50	1.50			
	II.	initial	pH	6.6	6.2	5.3	5.0	4.6	4.1
		final		5.1	5.0	4.6	4.3	4.3	4.1
		S/B_c		0.42	0.44	0.51	0.58	0.86	3.0

* Henceforth marked in the same way. C = control sample, 1, 2, 3, 4, 5 = lucerne treated with 2.5; 5.0; 7.5; 10.0; 12.5 lt^{-1} acid mixture (undiluted), respectively

For examination one jar of each treatment was opened after 0, 2, 4, 7, 15, 30, 60, 90, 120 and 210 days storage, resp., and, its midthird was equalized and analysed immediately.

1.2 Methods of microbiological analysis

The blending procedure of BINDER and WILHELM (1971) was applied for preparation of the silage samples. The total cell count was determined by plating on Plate Count Agar (PCA, Oxoid), the acid-producing useful microflora on modified Rogosa Agar (RA, Oxoid). The clostridial spore count was estimated by theMost Probable Number method in Reinforced Clostridial Medium (RCM, Oxoid). To identify silage microflora and for determining its properties the rapid identification pattern as designed by BECK (1972) was modified (Fig. 3). The sugar diagnostic analysis used for a more exact identification was developed (SZIGETI, 1976) from the "cylindric" agar plate method of KUNDART (1958). The basic medium was the Sugar Free Agar (SFA, Oxoid). For identification purposes we obtained some strains of lactobacilli by courtesy of T. DEÁK (University of Horticulture, Budapest).

1.3. Methods of chemical analysis

The pH values were determined in homogenized samples. The quantity of fermentable mono- and di-saccharides (S) was established (as % glucose) without partition by the method of MÖRTL (1972), and the integrated buffer

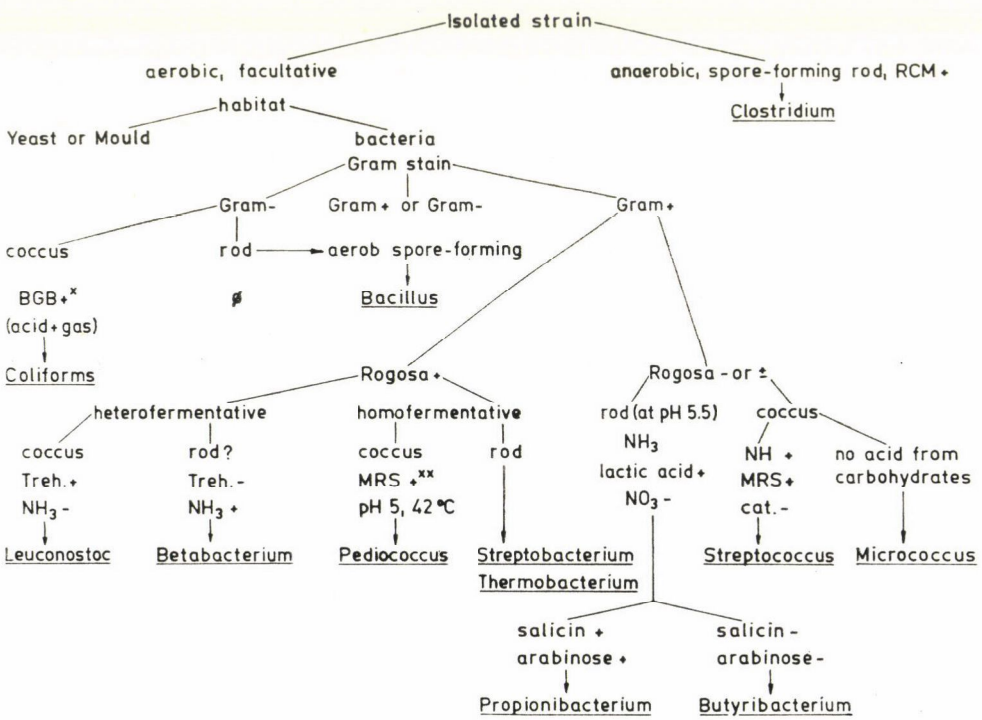

Fig. 3. Selection pattern grouped for identification of silage microflora

capacity (B_c) was examined according to WEISSBACH (1967). The gross composition was analysed according to Hungarian Standard (1972). For the quantitative and qualitative analysis of fermentation gases, we have designed (SZIGETI, 1976) an equipment. Equilibrium relative humidity was estimated by the method of VAS and CSONTOS (1956).

2. Results and conclusions

2.1. Occurrence of species of microbial strains identified in the main and post-fermentation periods

The taxonomical classification of the 147 microbial strains, selected at random from the strains isolated on the 7th day of the main fermentation period and during the post-fermentation period (on the 90th day) is shown in Table 3. The members of silage microflora converting, theoretically, at least 50% of the aldohexose into lactic acid under the conditions of silage preparation, were termed as useful flora. The microorganisms not accomplishing this criterion were classified as associated flora.

Data of the table indicate the lactic acid bacteria to give the major part of the total aerobic, microaerophilic and facultative anaerobic counts during the main fermentation period. The proportion of lactobacilli in the population increases with decreasing pH. This proportional increase is caused – as it was found also in earlier investigations – by an increase in the ratio of heterofermentative lactobacilli. With the lucerne mark I in silage treated with the two largest doses of acid, the microbial count constantly remained under the so-called "active" cell count limit. The cell count that produces a measurable amount of metabolites (organic acids, fermentation gases, ammonium-N) at 22 °C in a model silo of 0.1 m^3 in one day during fermentation is considered as active microbial count. The logarithm of this value (propagules per g) averages 7.85 during the post-fermentation period (period of decreasing microbial count). The measurement of the "activity limit" of the silage microflora was not attempted yet, but the data of PULAY (1967) relating to the gas-producing activity of pure clostridial cultures showed similar values. In the growth phase we could not separate the total microbial activity from that of the plant enzyme system, though, presumably, we should have obtained lower values in the early stages of fermentation.

During the post-fermentation period, in silages of high pH values, the proportion of homofermentative lactobacilli is low (under 10%), while at low pH, the ratio of homofermentative lactobacilli suffers only a small loss during 3 months. One factor of the decrease is the absence of pediococcus species. In the main fermentation period the homofermentative flora is fairly uniform

Table 3

Occurrence of species in the microflora of silages treated with various

Age of silage (days)	Symbol of treatment°	Useful microflora							
		Lactobacillus plantarum v. arabinosus	*Lactobacillus plantarum v. plantarum*	*Lactobacillus curvatus*	*Pediococcus cerevisiae*	*Lactobacillus previs*	*Lactobacillus cellobiosus*	*Lactobacillus buchneri*	*Leuconostoc mesenteroides*
7	C	3	1	1	1				1
	1	2	2		1	1	1		2
	2	2		1	3	2			2
	3	3	2		2	2	1	1	
	4	3	1		1	3		2	
	5	4	2			4			
90	C					3			1
	1				1	2			1
	2	2				3	1	1	
	3	2				3			1
	4	1				4		1	
	5	2				5			

(consisting of 1–3 species) and its most significant and most resistant representative is *Lb. plantarum* var. *arabinosus*. The proportional decline of pediococci, which is the slowest in grass silages (GOUET *et al.*, 1972), is considerable and their secondary growth in the post-fermentation period – mentioned by KROULIK and co-workers (1955) – may only be assumed in the case of Treatment 1. GIBSON and co-workers (1958) found *Lb. plantarum* and *Lb. acidophilus* to be dominant strains of the homofermentative flora. Considering that *Lb. acidophilus* has a temperature optimum substantially higher, than 22 °C, it is understandable why its isolation failed. From fresh and wilted grass silages, BECK (1972) isolated 7 homo- and 8 hetero-fermentative *Lactobacillus* species, among them, *Lb. curvatus* was present in the largest number (about 80% of the useful flora) early in the main fermentation period. Their count diminished to an insignificant value after 142 days of fermentation. The proportion of homofermentative strains isolated at the age of 8 days is in very good agreement with our findings as to the species isolated from lucerne. According again to BECK (1972), *Lb. mesenteroides* forms roughly the same ratio of the flora during the whole fermentation period. This appears to be

doses of acid during the main and the post-fermentation periods

Associated microflora								Number of identified strains	Total bacterial count (g^{-1})
Propionibacter zeae	*Butyribacter rettgeri*	*Coliforms*	*Micrococcus luteus*	*Micrococcus roseus*	*Streptococcus faecalis*	*Bacillus sp.*	Yeasts and Moulds		
1	1		1	1	1	1		13	$1.6 \cdot 10^9$
	1	1	1					12	$1.6 \cdot 10^9$
						2		12	$8.5 \cdot 10^8$
			1					12	$9.5 \cdot 10^8$
						1	1	12	$8.1 \cdot 10^6$
						2	1	13	$7.9 \cdot 10^5$
	2		1		5	1		13	$1.9 \cdot 10^8$
1	2		2		3	1		13	$1.8 \cdot 10^8$
			2		1	2		12	$9.5 \cdot 10^6$
			1		1	2	1	11	$2.1 \cdot 10^6$
						3	3	12	$2.6 \cdot 10^5$
						3	2	12	$1.1 \cdot 10^6$

* = see Table 2

confirmed by our data obtained in the control samples and those at the lowest acid dose. According to our investigations *Lb. brevis* dominating particularly at acid pH, is the leading strain of the heterofermentative flora. In addition to the above *Lb. cellobiosus* and *Lb. büchneri* appear to play an essential role in the main fermentation period. We found, however, that *Lb. mesenteroides* plays a definite role at extraordinarily low pH. It may be concluded in relation to the useful flora, that the reduction of the initial pH tightens the distribution by species of lactobacilli. At extremly low pH the lactic acid flora consists only of *Lb. plantarum* and/or *Lb. brevis*. In samples of high initial pH homofermentative lactic acid bacteria can hardly be isolated after 3 months. At the same time the heterofermentative flora undergoes a slight change only.

Two members of the *Propionibacteriaceae* family form the first group of associated flora. During the last 20 years GIBSON and co-workers (1958) were the pioneers who reported on the presence of propionibacteria in silages. The significance of these species lies in the fact that the formation of propionic or butyric acid would be possible not only under completely anaerobic conditions, moreover, that these acids could be formed in silages with low clostridial

counts. According to our experimental results the propagation of these species is, however, counteracted by low pH (approx. 4.2–4.6). At higher pH values, the "average lifespan" of these species is similar to that of the heterofermentative lactobacilli in silages. In the main flora coliform microorganisms were isolated in a single case only, thus, we did not consider its exact identification as necessary. As regards micrococci, a detailed account of isolation has not been published in the literature; they are followed down to families, only. BECK (1968), however, mentions a *Micrococcus pyrogenes* var. *albus* strain isolated from silage. In relation to the genus, he found their amount to remain below 20% of this total flora during the main fermentation stage. According to our data, their count is stable during fermentation. Their acid tolerance is rather high. *Str. faecalis* representing enterococci forms the main flora of the post-fermentation period in the cases of the control and lower doses of acid. In this control it was demonstrated during the main fermentation period, too. Several isolated strains were atypical, especially as regards lactose fermentation. Aerobic sporogenous bacilli were present in considerable counts in silages, and at about 40 °C, under aerobic conditions (GIBSON *et al.*, 1958; 1961), they can represent the main flora of silage. In these cases their counts in silages remain high also during the post-fermentation period. Practically, only a few species multiply under anaerobic conditions, but these, *e.g. B. licheniformis*, can be found also in silages (GIBSON *et al.*, 1958).

2.2. Initial multiplication rate of the useful and associated microflora

The rate of multiplication attained by the useful microflora immediately after silaging is very important since their proportion in the leaf surface microflora of plants is insignificant (WEISE, 1969). The reciprocal mean values of the generation times (h) obtained in our model silos as the function of the initial pH during the first 41.5 hours, are shown in Fig. 4.

As it may be seen, the multiplication rate of the useful flora is a multiple of that of the associated flora, even at high initial pH values. Although the multiplication rate of lactic acid bacteria also diminishes with decreasing initial pH, that of the associated flora decreases relatively more intensively. At a pH value of 4 the propagation of the deleterious flora will not take place at the beginning of the main fermentation period. At the same time this means that the pH value of 4 proffers protection against the growth of the associated flora up to 25% silage dry matter content, apart from the undissociated acid and other effects.

Practically, this also means that, if the sugar/buffer capacity coefficient is favourable for fermentation, the magnitude of losses arising in silages will have been decided at the beginning of the main fermentation period.

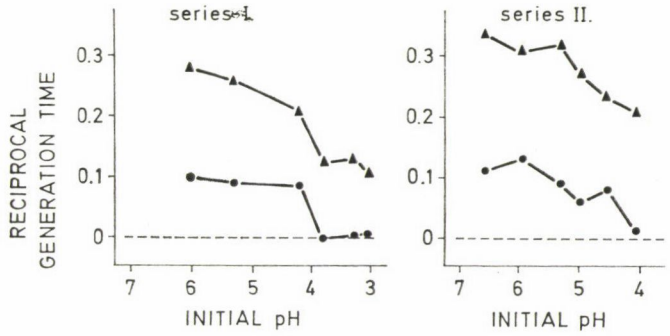

Fig. 4. Multiplication rates of the useful and associated microflora as a function of the initial pH in two different basic silage materials (Series I and II) treated with different doses of an inorganic acid mixture. (▲—▲ = useful microflora; ●—● = associated microflora)

2.3. Fermentation losses

First we will deal with the losses in dry matter being exhausted as fermentation gases. The fermentation losses in two experimental series as plotted against the initial pH values are demonstrated in Fig. 5.

Since the two kinds of basic material ensiled in these experiments may be considered two extremes from the aspect of their suitability, in the case of reducing the pH by adding acid, the loss through fermentation gases is characterized by the area between the two curves. It can be seen that acidifying to pH 5 will reduce the loss of dry matter to one half even in case the

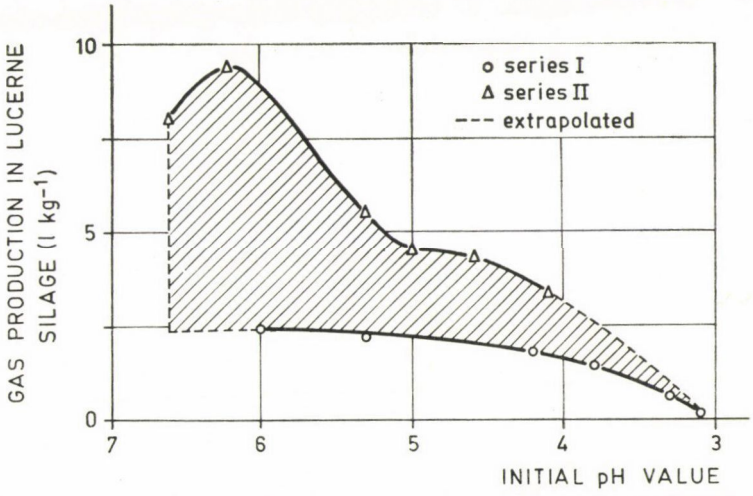

Fig. 5. Amounts of the gas developing during fermentation as a function of the initial pH values with two different basic silage materials (Series I and II)

basic material is unsuitable for ensilage, while acidifying to a pH of 4 results in reduction of losses to one third.

The other considerable source of losses is the breakdown of proteins and amino acids. The protein losses calculated on the basis of the ammonium content determined continuously from silages of 7 months or from the locking fluid of the gasometer are shown as percentages of the crude protein content in the basic material in Fig. 6.

As shown in the Figure, at relatively high initial pH the amount of ammonium nitrogen evaporating with fermentation gases can not be left out

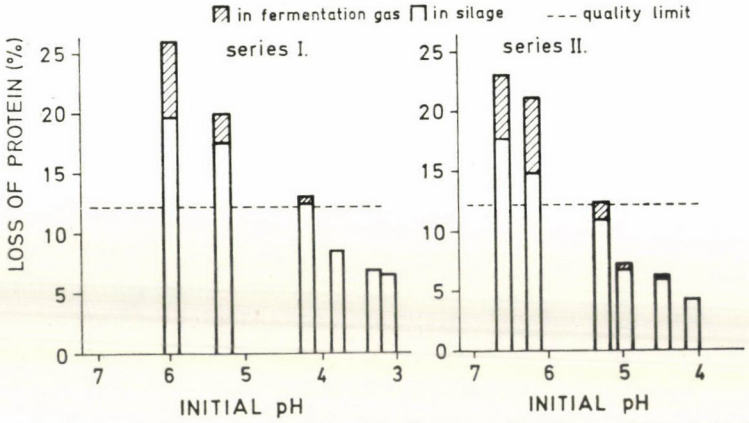

Fig. 6. Protein losses in the examined model silages as functions of the initial pH after an incubation period of 210 days

of consideration. Thus, the protein loss determined merely from the silage will not be accurate – especially with silages of poor quality. As shown by the results, de-amination in silages occurs at pH 4.2–4.6 while below this pH range losses of amino acids are due only to decarboxylation. It may also be seen, that the 12% loss accepted as the limit may also be fulfilled by acidifying to pH 4.

2.4. Clostridial spore count during fermentation

We shall deal separately with clostridial spore count not only because this microorganism is determined in a different way but, also because the saccharolytic Clostridia (particularly *C. tyrobutyricum* and *C. butyricum*) are the initiators of spoilage phenomena in pickled foods, in silages and hard cheeses. They transform lactic acid into butyric acid and are, therefore, of outstanding importance beside the lactic acid bacteria. Moreover, it is an important fact that the spreading of the application of monodiet in cattle feeding has made

silage feeding common and continuous all the year round. Thus, it is important that the silage fed to cattle in regions where hard cheese is manufactured should be poor in clostridia.

The clostridial count during silage fermentation is demonstrated in Fig. 7. The Figure shows data obtained in the IInd series. The buffer capacity of forage in this series is considerably higher, thus, the differences can be observed in a narrower pH zone more suitable for silage preparation. We did not differentiate between the spore counts of saccharolytic and proteolytic

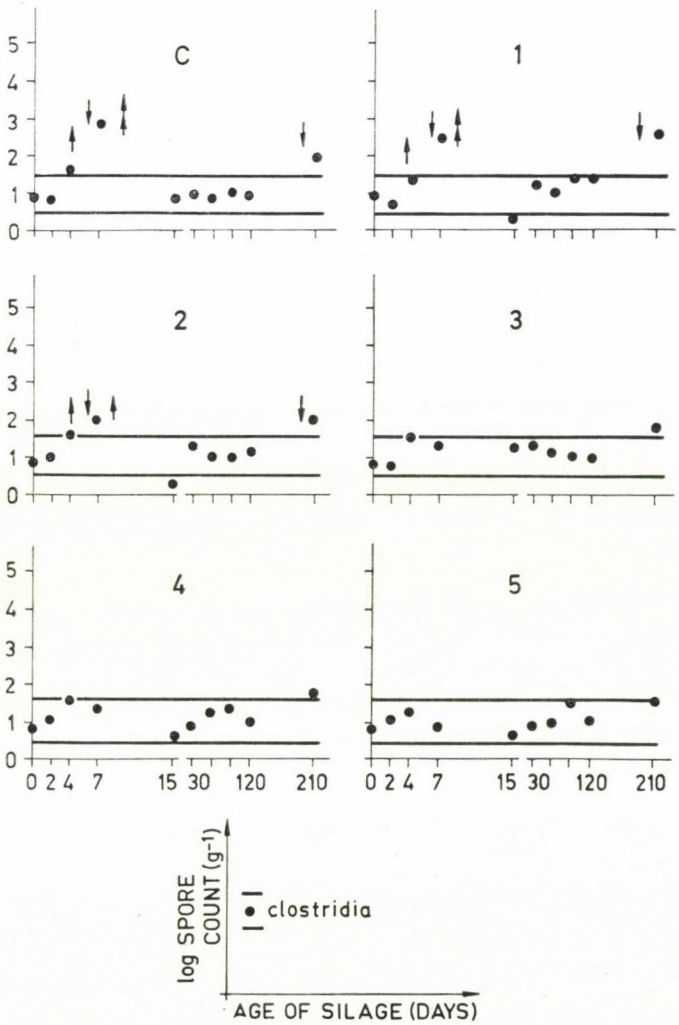

Fig. 7. The clostridial spore-counts as a function of fermentation time at different doses of acid C, 1, 2, 3, 4, 5 = mark of treatments (see: Table 2), ⎯ = experimental error, ↑ = spore germination and vegetative cell multiplication, ↓ = the start of spore formation

clostridia for it is a fact well-known from the literature that the spore count of saccharolytic clostridia will overlap the count of the latter in silages (GOUET *et al.*, 1972).

In evaluating spore counts the following viewpoints were considered: A *log* spore count fluctuation of ± 0.5 was considered not to exceed experimental error. If, within the same treatment, the spore counts increased significantly the only explanation would be that it has been preceded by vegetative cell multiplication and, at the given time, the essential conditions are unfavourable for vegetative cells. If the spore count decreases significantly, the spores must have been transformed into vegetative cells previously, and are in the state of multiplication. If the spore count increases gradually, the vegetative cells are progressively transformed into spores, which phenomenon is equivalent to the death phase of the non-sporogenous bacteria.

With the control sample the vegetative multiplication phase appeared to take place in two steps. There was a short multiplication phase between the 2nd and 4th day and this was followed by an intensive multiplication phase after the 7th day. A similar phenomenon was observed with the 1st treatment the only difference being that the final sporulation stage took place earlier, and the higher spore count noticeable in silages of 7 months could be interpreted by this fact. The data of the 2nd treatment suggest that the vegetative multiplication period had been limited to a shorter time in this treatment, and, presumably, the multiplication rate was slower, too. On addition of further doses of acid the rate of spore germination, outgrowth and propagation can be described as insignificant. It was found further that in the case of the I. series, (not shown here) the spore count had not been reduced significantly by the initial pH of 3.1 during 7 months. However, it may be undoubtedly stated that clostridia are not capable of considerable multiplication in forages acidified to pH values between 4.0 and 4.5. The contradiction well-known from the literature, *i.e.* that a low spore count may be found also in silages with high butyric acid content, can be resolved on the basis of the present investigations. This phenomenon is the consequence of the favourable living conditions for *Clostridia*, thus, they had been present for the most part in vegetative form, and upon uncovering the silage their destruction was brought about and their count could not be established by indirect microbiological methods.

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DEPENDENCE OF THE AMOUNT OF BOUND WATER OF FOODS ON TEMPERATURE

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The part of moisture in food which does not solidify upon freezing is bound water. The quantity of bound water may be numerically characterized by the monolayer value as calculated on the basis of the *BET* relation. Determining the sorption isotherms of freeze-dried beef, of starch solution and of raspberry at temperatures below freezing point and using these data to calculate the monolayer values it is found that the monolayer values increase in the region studied (20 –20 °C). It may be presumed that on further reduction of the temperature the monolayer value will equal the amount of water not yet frozen, because this value decreases with reduction of the temperature. The greater the quantity of the monolayer the higher is the temperature where the value of the quantity of monolayer and the amount of the non-frozen water become equal.

The term "bound water" is not defined exactly. Generally, it is understood to mean the part of the moisture content in foods the properties of which differ from those of pure water, (*e.g.* it is difficult to press out, more difficult to evaporate, it does not freeze at low temperatures, *etc.*).

Research into the structure of water and of aqueous solutions reveals the different ways of water binding, such as hydrogen, ionic, hydrophobic binding (Duckworth, 1975). However, it is essential to elucidate the effect and extent of the behaviour differing from that of pure water in the course of food processing. The determination of the amount of water not solidifying in the course of food freezing and the role of bound water in this phenomenon has been for long the subject of study and is partly known already. The elucidation of this problem is of great importance, because the quality of frozen foods depends largely upon the freezing of a part of the moisture content.

1. Review of the literature

1.1. Non-freezing water in foods

It is well-known that during the freezing of food a substantial part of the water contained in it turns into ice, however another part does not freeze even at a very low temperature. The part of the water not freezing may be considered "bound" water.

HEISS and recently RIEDEL (1972) studied, by determining the enthalpy and the freezing point of foods, the quantity of water not freezing in the course of freezing the food at different temperatures.

Some data, obtained by RIEDEL, appropriately transformed are given in Table 1.

Table 1

Moisture content and the quantity of water not freezing at various temperatures in some foodstuffs

Product	Moisture content x	Quantity of water not freezing at °C					Quantity of non-freezing water x_w
		−5	−10	−15	−20	−30	
		x_{wt}					
Lean beef	2.84	0.74	0.51	0.42	0.37	0.34	0.34
Fish (herring)	4.13	0.95	0.66	0.53	0.45	0.37	0.37
Strawberry	7.33	2.05	1.10	0.73	0.51	0.29	0.22
Peas	3.17	1.13	0.63	0.44	0.34	0.25	0.22

According to an earlier observation of RIEDEL at temperatures below −40 °C a perceptible amount of water does not get frozen in foodstuffs and he found that this water amounted to 0.2–0.4 g per g solids content.

RYUTOV (1976) subjected to critical examination the correlation suggested by NAGAOKA and co-workers (1955) and CHIZHOV (1966), based on *Raoult*'s law, for the determination of the ratio of water freezing in foodstuffs (total moisture content, $\omega = 1$)

$$\omega_j = 1 - \frac{t_{cr}}{t} \tag{1}$$

or

$$\omega_w = 1 - \omega_j = \frac{t_{cr}}{t}\,, \tag{2}$$

where

ω_j = the fraction of water freezing at different temperatures,
t_{cr} = the cryoscopic temperature (°C),
t = temperature below the cryoscopic point (°C),
ω_w = the fraction of water not freezing at different temperatures.

RYUTOV (1976) found that the values calculated by equation (1) for the proportion of non-frozen water are higher by 10–12% than those measured by RIEDEL.

He considers the cause of the difference to be the fact that NAGAOKA and coworkers (1955) and CHIZHOV (1966) did not account for the bound water present in foodstuffs.

1.2. The amount of bound water under freezing

According to RYUTOV (1976), on the basis of *Raoult*'s law, the ratio of frozen water is

$$\omega_i = \left(1 - x_b \frac{1 - W}{W}\right)\left(1 - \frac{t_{cr}}{t}\right), \tag{3}$$

where x_b = the proportion of bound water (g per g dry matter)
W = moisture content of the foodstuff, g per g product.

To determine x_b from the measured data the values belonging to ω_i are plotted in the system of coordinates $-1/t$, ω_i and the equation of the sample line $\omega_i = a + b(-1/t)$ is obtained. This, based on equation (3) and in the knowledge of W, gives, at the point $-1/t = 0$, the value of x_b.

Data pertaining to some foodstuffs as calculated by RYUTOV and partly by the author are given in Table 2.

Table 2

Total bound and non-freezing water and cryoscopic temperature in some foodstuffs

Product	x	t_{cr}	x_b	x_w
Lean beef	2.84	—0.95	0.257	0.34
Fish (herring)	4.13	—0.91	0.278	0.37
Strawberry	7.33	—1.2	0.147	0.22
Peas	3.17	—1.74	0.080	0.22

The data for bound water come close to, but are lower than those measured by RIEDEL at very low temperatures as non-frozen water.

Using the x_b values thus obtained in equation (3), the values as measured by RIEDEL at different temperatures for the non-frozen water, may be closely approximated (Table 3).

It may be seen in the Table that, taking into account the moisture content considered bound by RYUTOV and not freezable even at very low temperatures, the data calculated by equation (3) come closer to the measured data than those calculated by equation (2).

RYUTOV considers the quantity of bound water a constant value, independent of the temperature of freezing, however, not excluding the possibility of the freezing of a part of the weakly bound water with reduced freezing temperature. However, equation (3) does not permit of interpreting this.

RIEDEL (1961), on measuring the specific heat capacity with an adiabatic calorimeter down to –180 °C, gave a detailed analysis of free and bound water

Table 3

The measured and calculated value of non-freezing water in some foodstuffs

Product	Method	Water not freezing at °C				
		−5	−10	−15	−20	−30
		(g per g dry matter)				
Lean beef	measured	0.74	0.51	0.42	0.37	0.34
	calculated by Equ. (2)	0.54	0.27	0.18	0.14	0.09
	calculated by Equ. (3)	0.75	0.50	0.42	0.38	0.34
Fish (herring)	measured	0.95	0.66	0.53	0.45	0.37
	calculated by Equ. (2)	0.75	0.38	0.25	0.19	0.13
	calculated by Equ. (3)	0.98	0.63	0.51	0.46	0.40
Strawberry	measured	2.05	1.10	0.73	0.51	0.29
	calculated by Equ. (2)	1.75	1.02	0.59	0.44	0.29
	calculated by Equ. (3)	1.90	1.02	0.73	0.58	0.44
Peas	measured	1.13	0.63	0.44	0.34	0.25
	calculated by Equ. (2)	1.20	0.55	0.37	0.28	0.18
	calculated by Equ. (3)	1.15	0.62	0.44	0.35	0.26

in meat. The results of specific heat measurements in the range between −40 and −180 °C are given in Fig. 1.

It may be seen in the Figure that as regards specific heat at −180 °C, the simplest addition rule holds, the specific heat of the bound water is the same as that of ice. At −100 °C and a water content of 0.349 g per g dry matter, a break is visible showing that at this temperature this is the upper limit of the quantity of bound water. Assuming a rule of addition, the specific heat of bound water at −100 °C is 1.756 kJ $kg^{-1} K^{-1}$. At temperatures above −100 °C with water contents below 25.9% the rule of addition is not valid. According to the opinion of RIEDEL the increase in the specific heat of bound water is due to the desorption heat of bound water. Above the water content limit value of 0.349 g per g dry matter the rule of addition is valid, a higher water content may be considered free.

Knowing the specific heats of ice, bound and free water, and measuring the equilibrium relative humidity (ERH) of four beef samples of different moisture content, RIEDEL calculated, using the laws of *Raoult* and *van't Hoff*, the correlation between the moisture content and ERH of meat and the quantity of bound and free water in meat at different temperatures (Fig. 2).

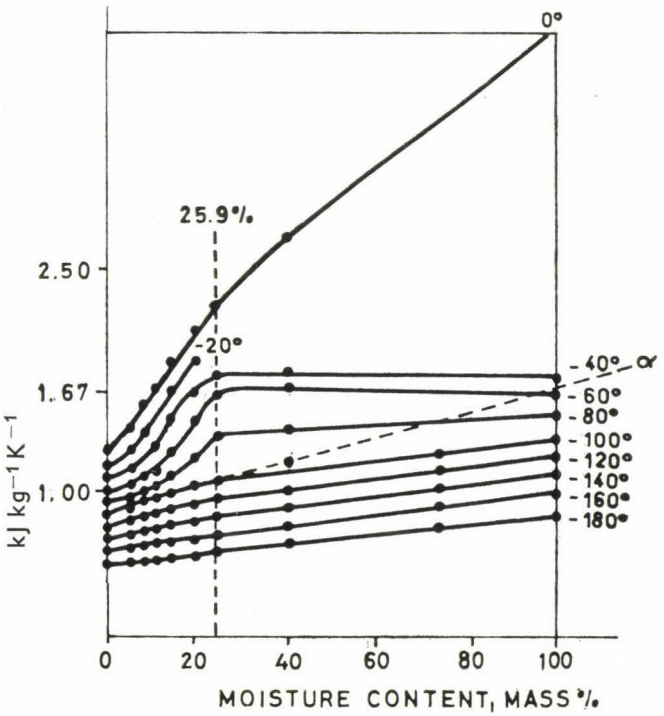

Fig. 1. Specific heat of beef of varied moisture content at various temperatures

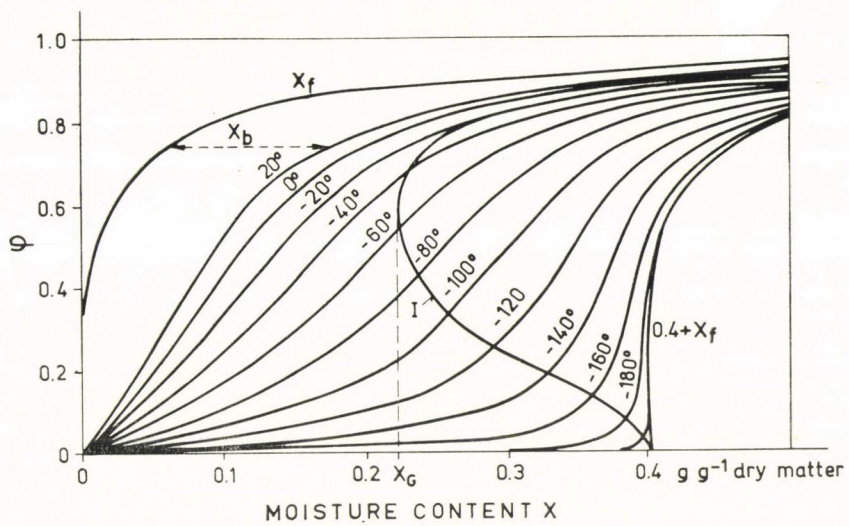

Fig. 2. Theoretical sorption isotherms of beef

With increasing temperature the amount of bound water decreases, x_b converging to 0. In the extreme case

$$x = x_f .$$

This is the limit curve on the left. By reducing the temperature, $x_b \rightarrow 0.4$, all positions of water binding will be occupied. The limit curve on the right is

$$x = 0.4 + x_f .$$

Marking the equilibrium relative humidities of the ice on the isotherms (the sublimation pressure of ice related to the tension of supercooled water) the ice curve (I) is obtained. This represents the actual right side limit of the isotherms. The ice curve related to axis x decreases up to a certain limit value, then it increases.

It may be concluded that at moisture contents lower than at the limit value of ice curve, ice is not formed even at very low temperatures.

Summing up the essence of the above, according to RYUTOV, reduction of the freezing temperature leaves the quantity of bound water constant: 0.26 g per g dry matter in beef. According to RIEDEL, the quantity of bound water increases with decreasing freezing temperature to a certain limit value and the maximum amount of bound water is 0.4 g per g dry matter.

Beyond the basic difference of the two view points their weakness lies in the fact that they are based on very few experimental data and the results were obtained mainly by calculation.

Therefore the aim of this work was set to clarify by experiments whether the quantity of bound water in beef and some other foods remains constant or increases with decreasing freezing temperatures.

1.3. The value of the BET monolayer in some foodstuffs

It is known that the condition of equilibrium of physical adsorption processes is described by the sorption isotherms. This is valid for the sorption of foods and food components as well.

Many authors were engaged in the mathematical interpretation of the sorption isotherms. Today the multilayer sorption theory of *Brunauer, Emmett* and *Teller* (*BET*) and the mathematical formula developed by them is considered an efficient method for the determination of the quantity of water bound to specific polar positions in dried foods (LABUZA, 1968). Although the *BET* process proceeds from highly simplified assumptions, the monolayer theory is useful because it may be correlated to the reduction of physical and chemical quality of dried foods.

The starting point in the multimolecular adsorption *BET* theory is that the first water layer is more closely adsorbed, its binding energy is greater

than that of the other layers and starting from the second layer the behaviour of water molecules is not different from that of free water.

Theoretically an unlimited number (n) of layers may be formed. In the case of W_0 amount of water belonging to a single layer, φ relative vapour pressure, c constant and assuming an infinite number of layers ($n = \infty$), the total adsorbed water quantity (W) is:

$$W = W_0 \frac{c\varphi}{(1 - \varphi)(1 - \varphi + c\varphi)}; \tag{4}$$

If $n = 1$, the layer is monomolecular:

$$W = W_0 \frac{c\varphi}{1 - c\varphi} \tag{5}$$

resulting in the *Langmuir* correlation.

The generally used form of the *BET* equation, derived from (4) ($W = x$ moisture content, $W_0 = x_m$ monolayer, $a_w = \varphi$)

$$\frac{a_w}{x(1 - a_w)} = \frac{1}{x_m \cdot c} - \frac{(c - 1)}{x_m \cdot c} a_w \tag{6}$$

If $x = f(a_w)$ sorption isotherm is used and

$$\frac{a_w}{x(1 - a_w)}$$

numerical value is plotted against a_w in a system of coordinates a straight line is obtained which at $a_w = 0$ intersects with the ordinate at value

$$I_i = \frac{1}{x_m \cdot c} \tag{7}$$

The slope of the line is given by

$$S = \frac{c - 1}{x_m \cdot c} \tag{8}$$

Since the above-described formulae of the *BET* equation do not account for the capillary effects, in the case of high *water activity* they are values suitable only to achieve a rough approximation. However, in the range $a_w < 0.35$, they give very good monolayer values.

With sorption isotherms of low constant c values (raspberry) another method was also used to determine x_m monolayer.

In the case of $x = x_m$, the relationship:

$$c = \left[\frac{a_{wm} - 1}{a_{wm}} \right]^2 \tag{9}$$

is valid, or in the case of a monolayer, the pertinent water activity (a_{wm}) and the c value are related. With low c value it is more expedient to use the latter calculation method, because equation (6) shows that the value of x_m is greatly affected if $c \to 1$.

IGLESIAS and CHIRIFE (1976) thoroughly investigated about 300 sorption isotherm data pertinent to food components available in the literature. Their results show that with decreasing temperature the value of the monolayer increases (Table 4). However, data published by these authors relate only to 5 °C

Table 4

The monolayer value in some foodstuffs as determined by the BET *equation*

Foodstuff (freeze-dried)	Method	Temperature (°C)	x_m (g per g dry matter)
Beef, raw	adsorption	10	0.069
		20	0.069
		30	0.064
		40	0.055
		50	0.045
Fish (herring), raw Back muscle	adsorption	5	0.079
		45	0.043
	desorption	5	0.088
		45	0.088
		60	0.035
Starch	adsorption	20	0.092
		30	0.082
		40	0.078
		50	0.072
Banana	adsorption	25	0.040
		45	0.031
		60	0.021

2. Materials and methods

2.1. Materials

The materials were selected to represent foods of protein, starch and sugar content.

Lean beef (leg) was purchased from the Budapest Enterprise of the Meat Industry. It was the meat of the breed *Magyar Tarka* (Hungarian Speckled), precooled for 24 h.

The potato starch used was a standard product of the Demecser Starch Factory.

The raspberries were of the variety *Malling Promise*, quick-frozen by the Hungarian Refrigeration Industry.

The samples, comminuted according to need, were freeze-dried on a *Metropolitan-Vickers* equipment at the Central Food Research Institute.

The residual moisture content of the freeze-dried samples was determined and with twice-distilled water their moisture content was adjusted to 0.1, 0.2, 0.3, 0.4 and with starch further to 0.5, 1 and 2 g per g dry matter, resp. The samples were then homogenized and, prior to analysis stored in ground-stoppered jars for a few days.

2.2. Methods

As it appears from the above the quantity of bound water may be characterized with numerical values by the monolayer as calculated from the sorption isotherms using the *BET* equation.

Preliminary investigations in relation to the sorption isotherms were carried out by the crystal liquefaction method (VAS & CSONTOS, 1956). According to ROCKLAND (1960) the results obtained by this method are nearly constant between 5 and 40 °C.

For the measurements, two methods were used in parallel: determination of the sorption isotherms above sulfuric acid solutions of different concentrations (WOLF *et al.*, 1973) and measurement of vapour pressure by manometer as suggested by LABUZA (1975).

In determining the sorption isotherms above sulfuric acid solutions, the dependence of vapour pressure on temperature (IMRE, 1974) was taken into account. To reach constant weight, 6–14 days were required.

The diagram of the apparatus for the measurement of vapour pressure by manometer is shown in Fig. 3.

The essence of the method is the measurement, by means of the manometer filled with an appropriate oil, of the vapour pressure built up above the food sample at the given temperature in the system evacuated to 0.27 mbar. The vapour was removed from the measuring space by a liquid nitrogen or dry ice condenser. The residual pressure is caused by non-condensing gases. The constant temperature of the sample was ensured by a low-temperature

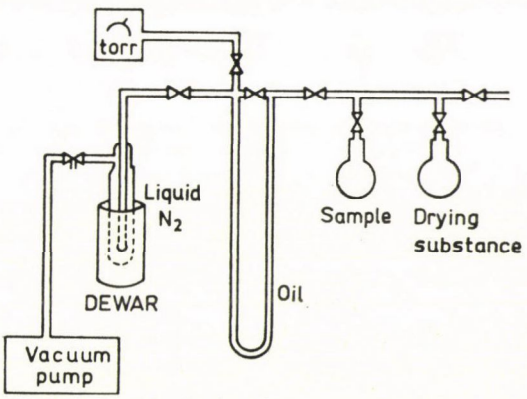

Fig. 3. Manometric device for measuring vapour pressure

ultrathermostat within the range of ± 0.2 °C. The very low residual pressure was measured with a GA 565-3 thermotron. The assembled apparatus is shown in Fig. 4.

The following temperatures were applied: 20, 5, 3, –5 and –20 °C. Measurements were carried out in 4–8 parallels. The results were evaluated by mathematical statistical methods.

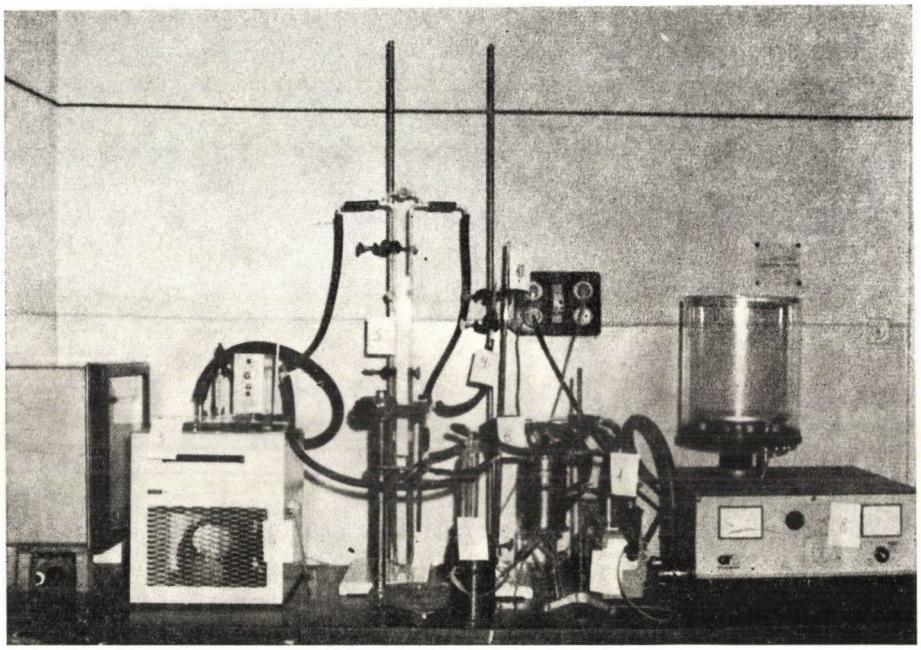

Fig. 4. Assembled manometric device for measuring vapour pressure. 1. Thermotron; 2. Container for alcoholic dry ice; 3. Material for investigation; 4. Ultrathermostat; 5. Oil manometer; 6. Vapour trap; 7. Vacuum pump; 8. Thermotron display; 9. Valve; 10. Mercury manometer

To calculate, from the sorption isotherms thus obtained, the value of the monolayer (x_m), equations 7, 8 and 9 were used. To determine I_i and S, the method of mean squares was applied.

3. Results

3.1. The sorption isotherms and the monolayer values

The desorption isotherm values, as measured above sulfuric acid solutions of different concentrations, and the monolayer values calculated are shown in Table 5. The desorption values obtained by vapour pressure measurement manometrically and the monolayer values calculated are given in Table 6.

On comparing the results obtained by the two methods these were found to give nearly identical results. However, the use of sulfuric acid led to more exact results suitable for use at −20 °C, thus in further calculations only these data were taken into account.

Table 5

Correlation between the moisture content and water activity in the materials investigated and the monolayer value at various temperatures

Temperature °C	Moisture content		Water activity a_w	Monolayer value g per g dry matter, x_m
	$\bar{x}$	s		
		Beef		
+20	0.060	0.006	0.05	0.082
	0.094	0.004	0.20	
+3	0.076	0.005	0.05	0.096
	0.111	0.001	0.20	
−5	0.091	0.002	0.05	0.103
	0.123	0.007	0.20	
	0.118	0.003	0.05	0.121
−20	0.149	0.006	0.20	
		Starch solution		
+20	0.140	0.004	0.05	0.164
	0.195	0.007	0.20	
+3	0.164	0.014	0.05	0.180
	0.218	0.018	0.20	
−5	0.212	0.014	0.05	0.210
	0.264	0.002	0.20	
−20	0.313	0.018	0.05	0.240
	0.319	0.025	0.20	
		Raspberry		
+20	0.063	0.005	0.05	0.080
	0.094	0.004	0.20	
+3	0.071	0.003	0.05	0.083
	0.099	0.001	0.20	
−5	0.101	0.002	0.05	0.097
	0.121	0.002	0.20	
−20	0.119	0.005	0.05	0.119
	0.147	0.007	0.20	

$\bar{x}$ = average of 8 measurements
s = standard error

Table 6

Correlation between the moisture content, vapour pressure and water activity on one hand and the monolayer value on the other at various temperatures in different foodstuffs

Temperature, °C	Moisture content g per g dry matter, x	Vaopur pressure mm of water		Water activity, a_w	Monolayer value g per g dry matter, x_m
		$\bar{p}$	s		
		Beef			
+20	0.1	110.8	4.44	0.46	
	0.2	83.8	18.9	0.35	
	0.3	118.2	9.7	0.48	
	0.4	131.0	12.2	0.55	0.07
+5	0.1	28.3	4.1	0.32	
	0.2	27.2	4.6	0.31	
	0.3	31.9	2.5	0.36	
	0.4	35.6	3.0	0.40	0.075
−5	0.1	13.0	1.6	0.32	
	0.2	18.9	1.0	0.45	
	0.3	17.9	5.2	0.49	
	0.4	22.9	0.8	0.56	0.10
		Starch solution			
+20	0.1	35.3	1.2	0.15	
	0.2	38.8	9.4	0.16	
	0.3	86.7	15.1	0.37	
	0.4	116.8	8.2	0.49	
	0.5	152.3	16.1	0.64	
	1	184.2	28.3	0.77	
	2	210.0	16.5	0.88	0.19
+5	0.1	15.4	0.7	0.17	
	0.2	21.2	3.4	0.24	
	0.3	39.7	1.7	0.45	
	0.4	60.5	6.7	0.68	
	0.5	65.9	4.4	0.74	
	1	76.6	6.2	0.86	
	2	82.7	2.2	0,93	0.25
−5	0.1	7.6	1.2	0.18	
	0.2	12.6	3.0	0.31	
	0.3	19.9	4.6	0.48	
	0.4	27.7	5.9	0.66	
	0.5	37.2	2.6	0.86	
	1	38.1	3.8	0.90	
	2	39.5	4.2	0.96	0.31
		Raspberry			
+20	0.1	93.8	8.6	0.39	
	0.2	109	11.2	0.46	
	0.3	124	11.2	0.52	
	0.4	133.8	16.5	0.56	0.05
+5	0.1	33.8	5.2	0.38	
	0.2	21.0	1.7	0.24	
	0.3	34.1	7.2	0.38	
	0.4	34.4	1.5	0.39	0.06
−5	0.1	18.5	3.2	0.45	
	0.2	19.3	1.7	0.47	
	0.3	25.8	2.5	0.63	
	0.4	2.04	1.9	0.50	0.08

p = average of 4 measurements
s = standard error

3.2. Comparison of the monolayer value and the quantity of unfrozen water in the foodstuffs studied

To determine the quantity of the non-frozen water, the freezing point values of the foodstuffs of different moisture content were used as obtained by thermoelements and differential thermal analysis (ALMÁSI, 1967) on one hand and the results obtained by RIEDEL (1961) as well as the data obtained by extrapolation on the basis of the correlation found by RYUTOV (1976), on the other. The values of the monolayer and of the non-frozen water are illustrated in Figs. 5, 6 and 7.

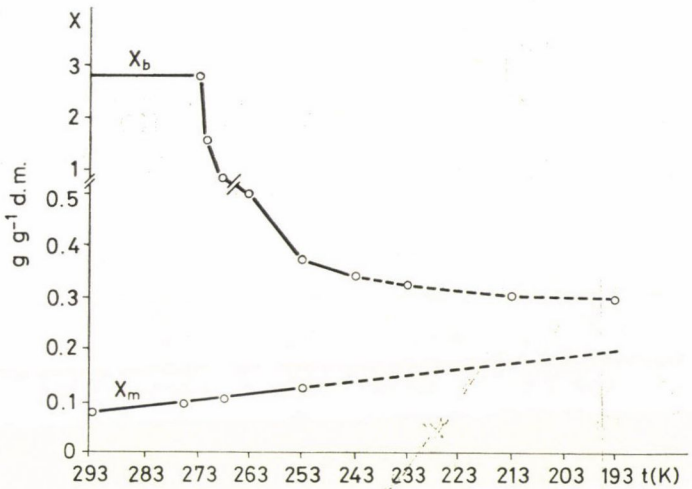

Fig. 5. The non-freezing moisture content of beef and the monolayer value as a function of temperature. $x_m = 0.36401–0.00097$ K; $s_y^2 = 0.0026$; $s_x^2 = 277.66$; $s_{xy} = \pm 0.00244$

In relation to the monolayer values correlation calculations were carried out with the data to determine their dependence on temperature.

The data extrapolated were marked by a dotted line.

3. Conclusions

The results of measurements and the Figures show that the monolayer value as established by the *BET* correlation increases in the foodstuffs examined at temperatures below the freezing point, thus the quantity of the bound water cannot be considered constant.

In the course of temperature reduction, the value of the monolayer probably reaches the quantity of the non-freezing water.

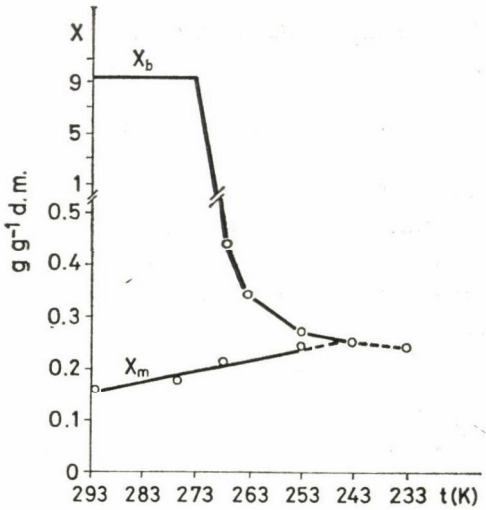

Fig. 6. The non-freezing moisture content in a starch solution and the monolayer value as a function of temperature. $x_m = 0.7326–0.00196$ K; $s_y^2 = 0.00113$; $s_x^2 = 277.66$; $s_{xy} = \pm 0.00967$

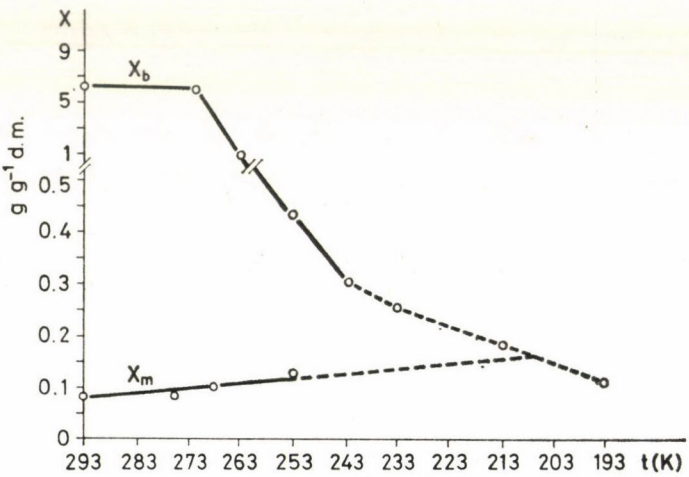

Fig. 7. The non-freezing moisture content of raspberries and the monolayer value as a function of temperature. $x_m = 0.24817–0.00056$ K; $s_y^2 = 0.00032$; $s_x^2 = 277.66$; $s_{xy} = \pm 0.01850$

With certain foodstuffs this temperature is relatively high (*e.g.* with starch solution: –30 °C), with others it is extremely low. Based on extrapolation, it is about –170 °C in beef.

With the starch solution, where the value of the monolayer and that of the non-freezing water meets at a relatively higher temperature, the value of the monolayer is much higher than with beef or raspberries.

Another characteristic feature of the starch solution is, according to the data in Table 6, that at identical moisture content and identical temperature, its water activity value is substantially lower than that of the other two foodstuffs studied or in other words the water is bound more closely to the starch.

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RAPID METHOD FOR THE DETERMINATION OF WILD YEAST CONTAMINATION IN BAKERS' YEAST BY LIPID ANALYSIS

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A relatively rapid and simple method for measuring wild yeast content of commercial bakers' yeast is described. Laboratory-scale experiments were carried out to obtain pure cultures of *Saccharomyces cerevisiae* and its most frequent infection, isolated and determined as *Candida utilis*. Artificial mixtures of dried biomass from yeast cultures were analysed chemically for lipids. Samples were taken in the course of the industrial process of bakers' yeast production in the factory. The effect of aeration rate on the infection of bakers' yeast was also studied in the laboratory to enrich the amount of wild yeast contamination in the biomass.

Yeast cells (about 5–10 g wet weight) were harvested by centrifugation, washed with HCl and distilled water, then dried at 378 K (105 °C) for 2 h. The finely ground dry material (0.5–1.0 g minimum) was saponified with 20% KOH in methanol water mixture. Fatty acids were extracted and analysed by gas chromatography as methyl esters. The precision of the whole procedure is reasonable, except for a complex process involving the errors of saponification, extraction and chromatography. *Saccharomyces cerevisiae* has no ability of synthesizing polyunsaturated fatty acids, whereas the proportion of linoleic and linolenic acids is very high (about 28–31%) in *Candida* species. In our method we expressed wild yeast contamination by the percentages of the linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acids in the total fatty acid content. The acceptable limit of the sensitivity of the chemical assay is about 4–5% wild yeast in the sample.

The quality control tests described in various standard prescriptions measure the properties of the yeast which are of interest to the consumer. The fermentative activity, keeping quality, consistency of the finished product are well defined by numerical parameters, but there is no technique for measuring microbial purity. Commercial bakers' yeast is not a pure culture, it may be more or less infected with bacteria, wild yeast (*i.e.* any yeast other than the strain being grown) and moulds. The wild yeasts are the most dangerous, since the nutrient media and cultivation processes for bakers' yeast are most suitable for them, too.

It is known that increasing content of wild yeast in bakers' yeast biomass causes decrease in the value of fermentative activity (*i.e.* less carbon dioxide is formed for doughing procedure in comparison with a noncontaminated yeast sample). Only one publication on the effect of wild yeast on the yield and quality of bakers' yeast (GONCHAROVA *et al.*, 1965) has been at our disposal so far. The authors proved, that the slowly reproducing species *Saccharomyces paradoxus* and *Torulopsis minor* inhibited the development of

bakers' yeast and reduced the yield, whereas *Candida utilis*, *Candida krusei* and *Candida mycoderma* increased the yield of bakers' yeast. It was further stated that the baking properties of industrial strains, their raising power, maltase activity and resistivity were greatly affected when infected with extraneous yeasts.

The detection, identification and counting of wild yeasts take 12 to 24 h before an estimate can be obtained (FOWELL, 1967). Even with the best selective media (MORRIS & EDDY, 1957; FOWELL, 1965; SCHERRER, SOMMER & PFENNINGER, 1969; LIN, 1974) it is rarely possible to estimate infection in less than 1–2 days. Tests for determining microbial infection of yeast bio-

Table 1

Different methods for wild yeast determination

Detection	Media	Author (date)
Selective media plating	lysine agar (brewery yeast) (bakers' yeast) crystal violet agar (malt)	MORRIS & EDDY (1957) FOWELL (1965) SCHERRER, SOMMER & PFENNINGER (1969)
	SDM (Schwarz-Differential-Medium)	LIN (1974)
Conductance or pH measurement	glucose-yeast extract-Tween 80	HARRISON, WEBB & MARTIN (1974)
Serological technique	immunofluorescent staining (*Saccharomyces* group)	RICHARDS (1969) RICHARDS & COWLAND (1967)
	(*Candida* and *Saccharomyces* groups)	HAIKARA & MAKINEN (1972) HAIKARA & ENARI (1975)
Chemical analysis of lipids and gas chromatography of fatty acids	dried biomass	BIACS (1977)

mass are rather time-consuming, especially for wild yeasts. Several methods have been published for the detection of wild yeasts, such as the selective plating procedure (lysine agar, crystal violet agar, Schwarz Differential Medium) or examination of the conductance and pH in the medium (HARRISON *et al.*, 1974). The immunofluorescent detection proved to be most suitable as a routine quality control method in the brewery (RICHARDS, 1969; RICHARDS & COWLAND, 1967) and the practical value of the technique has also been established (HAIKARA & MÄKINEN, 1972; HAIKARA & ENARI, 1975) (Table 1).

The lipid composition of different yeast strains is known in great detail (KANEKO *et al.*, 1976). In *Saccharomyces cerevisiae* the most abundant fatty acids are monoenic (palmitoleic $C_{16:1}$ and oleic $C_{18:1}$) acids. (HUNTER & ROSE,

1972). It is well established that *Saccharomyces cerevisiae* has no ability of synthesizing polyunsaturated fatty acids. Our previous experiments (BIACS, 1974; BIACS & HOLLÓ, 1977) involving lipid analysis of different yeast species showed a little occurrence of $C_{18:2}$ and $C_{18:3}$ polyene fatty acid components in the percentage of total fatty acid content of *Saccharomyces cerevisiae*. The proportion of linoleic and linolenic fatty acids is usually very high in *Candida* species (RATLEDGE, 1970) and they could be used for future supplies of oils and fats produced microbiologically.

Fatty acids, as the main components of yeast lipids, are characteristic of yeast classes, as well (Table 2).

Table 2

Main fatty acids of Saccharomyces cerevisiae *and wild yeasts in bakers' yeast*

Yeast species		Main fatty acids in the fatty acid composition, %					Polyene fatty acids, %
		$C_{16:0}$	$C_{16:1}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	
Saccharomyces cerevisiae	(a)	15.6	43.4	26.9	—	—	—
	(b)	8.9	57.0	30.3	1.5	—	1.5
	(c)	15.6	25.8	37.5	2.0	0.8	2.8
Candida krusei	(d)	15.0	6.2	48.2	14.9	13.7	28.6
C. tropicalis	(d)	21.8	5.4	28.6	26.2	4.4	30.8
C. pulcherrima	(d)	21.0	7.4	41.4	25.6	1.4	27.0
C. utilis	(b)	23.8	16.5	19.6	23.9	5.2	29.1
Candida 107	(e)	21.0	3.0	36.0	28.0	—	28.0
Pichia membr.	(d)	12.3	14.8	40.9	23.6	7.7	31.3
C. mycoderma	(d)	14.1	17.1	41.1	18.6	4.7	23.3
Torulopsis cand.	(d)	27.9	3.7	42.5	11.9	2.7	14.6
Rhodotorula rubra	(c)	22.2	—	61.0	10.2	2.7	12.9

Note: (a) HUNTER & ROSE (1972) continuous cultivation
(b) BIACS (1977) pure bakers' yeast from factory
(c) BIACS & HOLLÓ (1977) laboratory-scale cultivation
(d) KANEKO *et al.* (1976) plating on YM-agar medium
(e) RATLEDGE (1970) cultivation on glucose

In *Saccharomyces cerevisiae* the polyunsaturated fatty acids were determined to be always under 3%, whereas in most *Candida* species the figures were between 28 and 31%. Thus we suggest that this purely chemical analysis be used instead of biological and physical methods for wild yeast determination in bakers' yeast culture (BIACS, 1977).

1. Materials and methods

Yeasts used in this study originated from the collection of the Hungarian alcohol factories. Pure cultures of *Saccharomyces cerevisiae* and *Candida utilis* were maintained on slants of malt agar. Both yeasts were grown in the labora-

tory on "synthetic" molasses medium. Artificial mixtures of dried biomass from both yeast cultures were analysed chemically for lipids, together with commercial bakers' yeast samples taken from the Budafok Yeast Factory.

1.1. Yeast proliferation

Synthetic "molasses" were prepared as follows: 50.0 g sucrose, 7.0 g $(NH_4)_2SO_4$, 1.5 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.1 g NaCl, 0.1 g $CaCl_2 \cdot 2H_2O$ and 5.0 g yeast extract (DIFCO) were dissolved in 1000 ml tap water, then the pH adjusted with 25% H_2SO_4 to 4.5. Sterilization was carried out in an autoclave at 393 K (120 °C) for 2 h. The sucrose–nitrogen–phosphorus ratio corresponded to the nutrient used in the production fermenters in the factory.

For batch cultures, 200-ml portions of medium were dispensed into 750-ml Erlenmeyer flasks, sterilized and inoculated. Shaken flasks were incubated at 303 K (30 °C). Laboratory-scale cultures were grown in 10.0-l glass fermenters fitted with devices for control of temperature and pH. All parameters were kept under similar conditions, the aeration rate however ranging from low, medium to high volume of inlet sterile air.

Samples taken from the factory were more or less infected with wild yeast, as shown by different detection methods (plating on selective media, microscopic investigation, *etc.*). To enlarge the ratio of wild yeasts in the biomass commercial samples were taken as inocula. Samples of slightly contaminated bakers' yeast (wild to culture yeast ratios of 1 to 60 or to 20) were inoculated into fresh sterile media, incubated for 20 h in shaken flasks, then a 1 : 10 scale-up culture transferred into glass fermenters for biomass production. In a parallel run, with 3 fermenters in a battery, variations were made in the aeration parameters, when the inlet air was ranging from zero, 1.0 and 2.0 l min^{-1} calculated to one liter liquid medium. Reaching the declining phase of the growth rate curve in 20 h, yeast cells were separated by centrifugation and analysed as above.

Yeast strains isolated by plating and developing colonies from commercial bakers' yeast samples were also propagated.

1.2. Growth kinetic measurements

It is well known that *Candida* species are growing more rapidly than *Saccharomyces* cultures.

To follow the change of cell concentration reliably, cells were counted under the microscope and the optical density of original and diluted samples was measured turbidimetrically. The growth of yeast populations has been characterized by the growth rate curve (BIACS *et al.*, 1974). The aim of growth

kinetic measurements was to obtain numerical data for the growth rate coefficient "k" and the profile of the growth process (the number and character of phases) as illustrated in Fig. 1 for *Saccharomyces cerevisiae*.

1.3. Lipid analysis

For lipid analysis yeast cells were harvested by centrifugation and washed with diluted hydrochloric acid and distilled water. The obtained yeast biomass was dried at 378 K (105 °C) for max. 2 h. The fatty acid content of

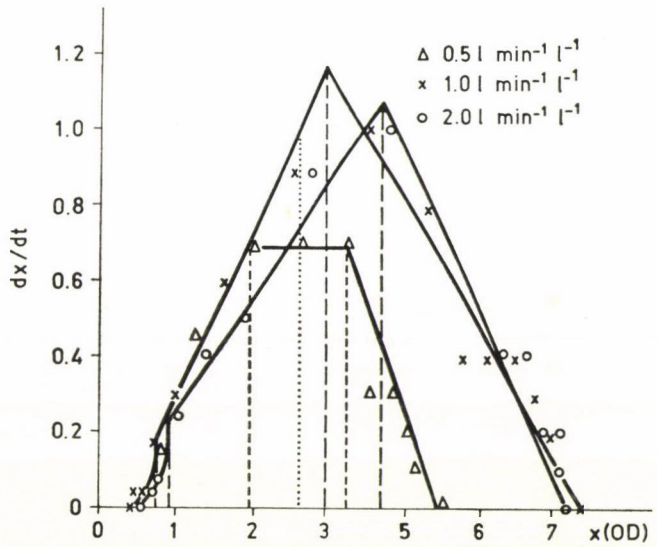


Fig. 1. Growth rate curves of *Saccharomyces cerevisiae* in different aeration conditions: low (○), medium (x) and high (0) inlet sterile air. "k" values: 0.45 ($k_{0.5-1.0}$) and 0.35 ($k_{2.0}$)

yeast samples was obtained by direct saponification of finely ground dry yeast (minimum 0.5–1.0 g) with 20% KOH in methanol–water (1 : 1). The procedure resulted in decomposition of lipids into saponifiable, non-saponifiable and water-soluble parts. Non-saponifiable, neutral components (hydrocarbons, sterols, *etc.*) were removed by extraction with petroleum ether and diethyl ether mixture (1 : 1) then analysed by thin-layer chromatography.

Fatty acid soaps were acidified and then extracted with the same solvent mixture. The fatty acids obtained were converted into methyl esters for gas chromatography by STAHL's method (1969).

Gas-chromatographic determination of fatty acid composition. The distribution of fatty acid methyl esters was carried out by different gas-chromatographic measurements. Detailed analysis was done by capillary columns coated with *Carbowax* 20 M according to SCHOMBURG and HUSMAN (1975).

A 25 m long and 0.25 mm i.d. glass column was fitted in a *Carlo Erba 2450* gas chromatograph (linear flow rate: 27 cm s^{-1}) then a 1.0 μl sample was injected (a 1/25 split at 0.5 atm inlet, septum flush 2 ml min^{-1}). Thus the analysis resulted in a dead volume of 1.5 min. Separation was carried out at 433 K (160 °C) column temperature and gave 30–35 fatty acid peaks in yeast lipids (Fig. 2).

For routine analysis, a more polar chromatographic phase, 10% diethylene glycol succinate (DEGS) polymer supported on 100–120 mesh *Chromosorb* W, was used for determining C_{18} polyunsaturated fatty acids. A 2.4 m long glass column (2.5 mm i.d.) was fitted in a gas chromatograph model *Chrom-31*, equipped with a flame ionization detector (FID), thus ensuring isothermic conditions at 458 K (185 °C).

Quantitative analysis of the main components was established by measuring peak areas in the chromatogram by triangulation. Fatty acid composition was determined in percentages of total peak area. Capillary column chromatography was recorded by a *Finnigan* 3000 GC-MS Data Systems and peak area calculated by an electronic integrator.

2. Results

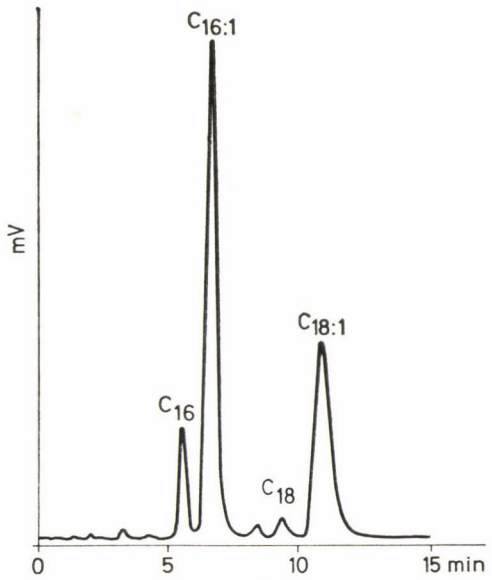
The fatty acid composition of yeast lipids showed a predominance of C_{16} and C_{18} monounsaturated acids for *Saccharomyces cerevisiae* and commercial bakers' yeast samples. Data for pure bakers' yeast *(Saccharomyces cerevisiae)* and slightly contaminated samples of the factory are summarized in Table 3, together with its most frequent infection, isolated and determined as *Candida utilis*.

Table 3

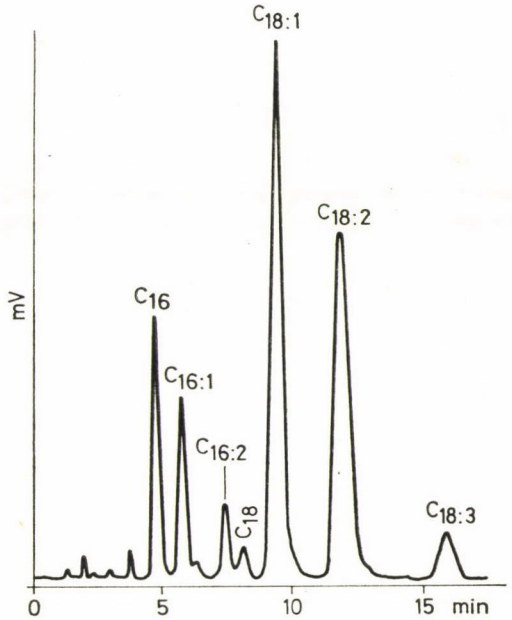
Fatty acid composition (means and standard deviations) of different yeast samples from a bakers' yeast factory

Fatty acids	Pure baker's yeast[a]	contaminated samples[b]	Isolated wild yeast[c]
$C_{14:0}$	traces	0.3±0.1	2.8±0.2
$C_{16:0}$	8.9±0.5	12.4±0.7	23.8±0.8
$C_{16:1}$	57.0±1.4	55.0±1.2	16.5±1.1
$C_{16:2}$	—	0.8±0.1	6.2±0.2
$C_{18:0}$	2.2±0.3	2.1±0.4	1.9±0.3
$C_{18:1}$	30.3±1.8	23.9±1.7	19.6±1.0
$C_{18:2}$	1.5±0.1	5.6±1.1	23.9±0.8
$C_{18:3}$	—	1.8±0.4	5.2±0.1

Note: [a] BIACS (1977)
[b] average of 5 different samples being contaminated with 3—5% wild yeast as determined on lysine agar
[c] isolated on lysine agar and proliferated in the laboratory



a)



b)

Fig. 2. Gas chromatogram of yeast lipids. Conditions: *Chrom–31* FID 10% DEGS on 100–120 mesh *Chromosorb* W, 2.4 m glass column (2.5 mm i.d.) 458 K (185 °C). Abbreviations: C_{16} – palmitic acid; $C_{16:1}$ – palmitoleic acid. a) Fatty acids of *Saccharomyces cerevisiae*. b) Fatty acids of *Candida utilis* (wild yeast)

Under the same circumstances of yeast proliferation on a laboratory-scale cultivation on "synthetic" molasses (only saccharose), *Candida utilis* cells were slowly growing because of their little invertase activity. The obtained biomass contains about the same quantity or less of total fatty acids than *Saccharomyces cerevisiae* cells (1.6–1.8% and 1.7–1.9% resp.). Thus the qualitative differences in the fatty acid composition were less influenced by quantitative deviations, when the biomass of both pure cultures was mixed by weight.

Artificial mixtures of 25%, 50% and 75% *Candida utilis* dry biomass to *Saccharomyces cerevisiae* (data in Table 3) were analysed. The amount of $C_{18:2}$ and $C_{18:3}$ fatty acids in percentages of total fatty acids were plotted in a diagram against wild yeast concentration by weight in a mixture, as illustrated in Fig. 3.

Enrichment of wild yeast in a sample. To enlarge the amount of wild yeasts in commercial bakers' yeast samples and to increase the sensitivity of the gas-chromatographic method, laboratory-scale cultivations were carried out with differently contaminated samples as inocula. The amount of "wild" yeast *(Candida utilis)* could be well-established from 5% upward in commercial bakers' yeast samples using Fig. 3 as a calibration curve. Less infected nocul a (1 : 60) resulted in wild yeast contents between 5–20%, depending

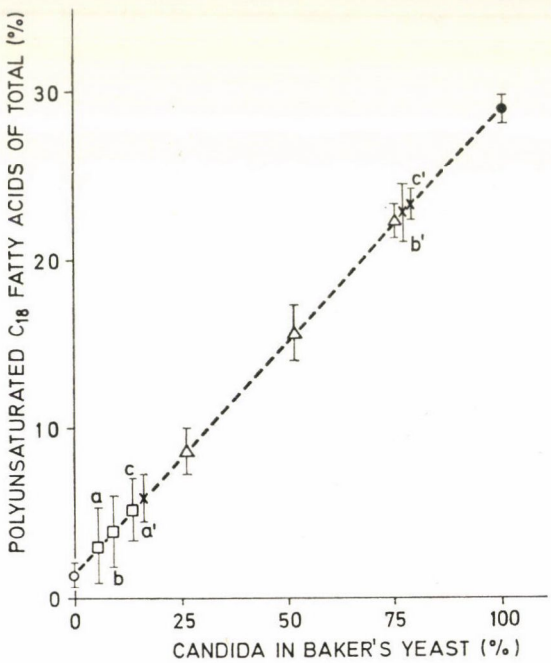


Fig. 3. Results of gas chromatographic analysis: calibration. (○) sterile propagated *Saccharomyces cerevisiae*. (●) sterile propagated *Candida utilis*. (○) artificial mixtures: 25%, 50% and 75%. (○) a–b–c biomass of less infected inocula. (○) a'–b'–c' biomass of more infected inocula

on aeration rate applied in the fermenter (a-b-c), whereas more infected inocula (1 : 20) caused more enriched (78–80%) wild yeast content when aerated intensively in the process (b'-c').

Gas-chromatographic results of the original a' sample (1 : 20), enriched culture and both isolated strains are illustrated in Fig. 4.

It is well-known that, at low aeration and consequent high alcohol concentration in the fermentation media, *Saccharomyces cerevisiae* is less infected with wild yeasts. By the help of fatty acid analysis it could be numerically stated that, under those circumstances, only the growth of the pure

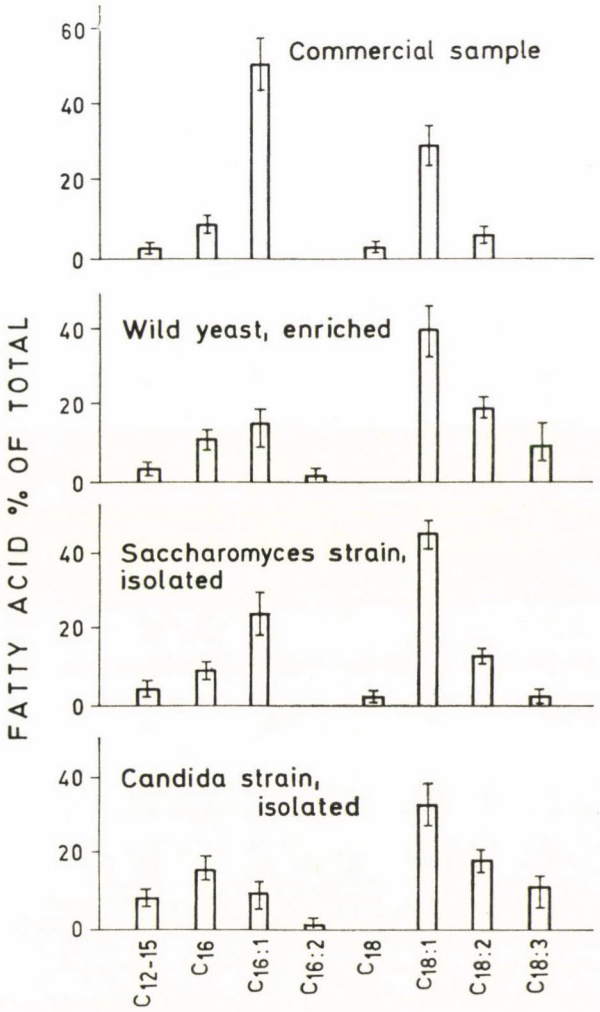


Fig. 4. Fatty acid distribution of different yeast samples (means of 3 parallel runs, standard deviations)

bakers' yeast strain was increased and the development of wild yeast infection was hindered. At medium and high inlet of sterile air wild yeasts proliferated to a great extent and were detected in the culture.

3. Conclusion

The method of determining wild yeast contamination with this purely chemical analysis of the dried bakers' yeast biomass proved to be a rapid and reproducible one. Compared with biological methods, like using selective media or immunofluorescent staining, this method seems to be more reliable. Sensitivity and reproducibility can be elaborated in the next step by detailed chemical analysis and the original sample can safely be stored in the dried form. We are convinced that this method will be of interest to microbiologists to use this instrumental analytical method for biological determination.

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We wish to express our thanks to Ms. A. Barkóczay, chief-engineer of the Budafok Yeast Factory, who kindly permitted the taking of samples in the plant.

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THE DETERMINATION OF THE IRON/CHELATING ABILITY OF DIFFERENT CARBOHYDRATES AND THE PREPARATION OF FERRIC/CARBOHYDRATE COMPLEXES

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A series of carbohydrates are compared in their ability to chelate ferric iron at alkaline pH (pH 9) in aqueous solution. Hydrogenated or reduced carbohydrates (sugar alcohols and their derivatives) are shown to possess a greater ability to chelate iron in aqueous solution than their unmodified counterparts. In agreement with previous work, fructose is shown to possess the greatest chelating ability amongst the carbohydrates studied.

The preparation, isolation and analysis of a series of ferric/carbohydrate chelates is described and the results of the analyses confirm in part the previously proposed structures of the chelates. It is shown that although a carbohydrate apparently chelates a large quantity of iron in aqueous solution, this is not necessarily reflected in the analysis of the isolated chelate. For example, sorbitol apparently chelates more iron in solution than glucose, but when the respective chelates are isolated, the glucose chelate contains a greater proportion of iron.

Finally, the initial carbohydrate concentration is shown to influence the chemical composition (especially iron content) of the isolated chelates. The lowe the initial carbohydrate concentration the greater the amount of iron in th isolated chelate, over the range studied.

The chelation of alkali and alkaline earth metal salts by carbohydrates is well-known (RENDLEMAN, 1966a, 1966b) and the importance of selected chelates in the diet has been established (LAYRISSE *et al.*, 1976). We have previously described the importance of iron in the human diet and alluded to the literature regarding the increased absorption of iron when it is taken in conjunction with carbohydrate compounds and more especially fructose (KEARSLEY & BIRCH, 1977a). Ferrous sulphate is often used in the treatment of anaemia in humans, the ferrous iron being oxidized to ferric ion after absorption into the body from the gut. Ferric iron is not transported across the mucosal wall and thus is not available to the body if administered orally. Sorbitol and fructose have been shown to enhance the absorption of ferric iron into the body (CHARLEY *et al.*, 1963a) and it has been reported that ferric/fructose provides a more easily absorbable form of iron than ferrous sulphate (BATES *et al.*, 1972). It is generally agreed that fructose is probably the best carbohydrate chelating agent for ferric and ferrous iron and whilst many iron/fructose investigations have been reported (CHARLEY *et al.*, 1963a, b; BARKER *et al.*, 1974; AMINE & HEGSTED, 1975) little work has been directed to the formation and isolation of chelates using other carbohydrates. Soft drinks

have been suggested as iron carriers since their high carbohydrate content will chelate iron in relatively large concentrations. Ferric iron is known to form chelates more easily than ferrous iron and difficulties have been found when attempting to isolate ferrous/fructose chelates owing to the rapid oxidation of ferrous to ferric iron (LAYRISSE *et al.*, 1976).

Chelation of iron by carbohydrates does not necessarily mean better absorption into the body and obviously feeding studies would have to be carried out on new chelates. Ferric-EDTA chelates are for example very poorly absorbed (CHARLEY *et al.*, 1963a). Chelation of iron also has industrial significance, more especially when carbohydrate solutions come into contact with various steel construction materials. Any iron dissolving in the solution may then be available for chelation by the carbohydrate.

We have thus restricted our present study to the use of ferric iron and the comparison of new ferric carbohydrate chelates with ferric fructose chelates. The study presents our preliminary results on the properties of ferric carbohydrate chelates and deals with the qualitative production and isolation of these chelates and their quantitative analysis. It is envisaged that these newer chelates could possibly find uses in the treatment of anaemia for example, although their effect on iron uptake in the body would first have to be established.

1. Experimental

1.1. Materials

Glucose syrups and D-glucose were provided by Corn Products Ltd., Manchester, England (courtesy of Dr. D. HOWLING). High fructose glucose syrup (HFGS) was provided by Tunnel Refineries Ltd., Dartford, England (courtesy of Dr. T. PALMER). Both these are gratefully acknowledged. Hydrogenated glucose syrups, sorbitol and maltitol were produced by a previously described technique (KEARSLEY & BIRCH, 1977b).

Xylose, xylitol and maltose were obtained from Sigma Chemicals Ltd., London, England, and sucrose, fructose and lactose from BDH Chemicals Ltd., Dorset, England.

1.2. Methods

1.2.1. Concentrations of ferric iron and carbohydrate for chelation. It is important to quantify the chelating ability of carbohydrates with respect to iron. Initially, we added aqueous ferric nitrate and sodium hydroxide simultaneously to a solution of the carbohydrate to maintain the pH between

8.5 and 9.0 until a precipitate of ferric hydroxide was observed. No precipitate occurred as long as the ferric iron was chelated and effectively removed from solution. The precipitate was taken as the "end point" of the reaction when no more iron could be chelated. This method proved totally unsatisfactory, however, since the rate of addition of the iron and the alkali to the carbohydrate governed the end point. No satisfactory method based on this principle could be devised because of the number of variables involved, *i.e.* time of reaction, rate of stirring and sugar configuration (open chain or ring). The method of CHARLEY and co-workers (1963b) was therefore used with slight modification. (A more elaborate method based on this procedure has also been described whereby radioactively labelled iron in solution was measured after mixing carbohydrate and iron salt – DAVIS & DELLER, 1966.) The modified method was considered sufficiently accurate for a rapid characterization of the chelating ability of different carbohydrates.

A 1.0 *M* solution of ferric nitrate and a 60% solution of each carbohydrate were prepared. These were mixed in predetermined proportions and combinations such that six dilutions of carbohydrate (50, 40, 30, 20, 10 and 5% w/v in the final concentration) had each been mixed with eight dilutions of the iron salt (0.2, 0.1, 0.08, 0.06, 0.04, 0.02, 0.01 and 0.005 *M* in the final concentration), giving a total of 48 interactions.

Each solution was titrated quickly to pH 9 with 10 *N* sodium hydroxide and any precipitate noted at the alkaline pH *immediately* after mixing (*N.B.* if the samples are left to stand for some time the precipitates in some cases dissolve). The rate of addition of alkali obviously affected the result but providing the same method was used for each sample, reproducible results were obtained. In previous determinations any precipitate over the whole pH range was taken to indicate that total chelation had not occurred but in many cases, especially at higher iron concentrations, precipitates are formed between pH 4 and 7 which redissolve above pH 7 and remain stable up to pH 14. In this present study "iron numbers" have been assigned to each carbohydrate based on the number of successful chelations at the various interaction concentrations of iron and carbohydrate. Thus fructose chelated the added iron successfully (no precipitate) at all but the two highest iron concentrations and the lowest fructose concentration (2 interactions) and thus 46 out of 48 interactions were negative. Fructose was therefore assigned an iron number of 46.

1.2.2. Preparation, isolation and properties of ferric/carbohydrate complexes. A 50% w/v solution of each carbohydrate was prepared and 25 ml placed in the reaction vessel. A 1.0 *M* solution of ferric nitrate and 3.0 *M* solution of sodium hydroxide were prepared and transferred to 50 ml burettes. The ferric nitrate and sodium hydroxide were added slowly and simultaneously to the carbohydrate to maintain the pH between 8.5 and 9.0 (CHARLEY *et al.*, 1963b; BARKER *et al.*, 1974) until either 25 ml of the ferric

salt had been added or a precipitate of ferric hydroxide started to form. In this latter instance continued stirring usually dissolved the precipitate and if this was not successful the experiment was repeated. In all cases the colourless carbohydrate solution turned yellow, then orange, through brown, finally being black. Absolute alcohol was added to this black solution to 60% by volume and the complex was precipitated. This was the first precipitation stage. Two types of first precipitate were found and these will be referred to as "glucose type" and "fructose type" precipitates. The fructose type were extremely viscous and sticky and settled as a sludge in the mixing vessel. The supernatant could be poured off and the precipitate redissolved in water. Glucose type precipitates were very fine and formed a suspension which required centrifuging to collect the chelate.

After redissolving the precipitates, a second alcohol precipitation was carried out to purify the chelate. All second precipitates were of the glucose type. The chelates were collected after precipitation, dried at 60 °C under reduced pressure and stored over P_2O_5 until required for analysis. Glucose, sucrose and xylose formed glucose type precipitates whilst the remaining carbohydrates produced the fructose type.

1.2.3. Analysis of chelates. All analyses of chelates were carried out on the dried, isolated product.

Moisture was calculated as the percentage loss in weight when 1 g of the chelate was dried for 3 hours at 100 °C.

Ash was calculated as the residue remaining after 1 g of chelate was ignited at 500 °C for 6 hours. (During the preliminary ashing procedure the chelate was first ignited using a Bunsen burner. These compounds burn exothermically and require only a preliminary ignition to start the reaction, of iron filings and sulphur.)

Iron. Ferric iron was determined by the thiocyanate method on the acid soluble part of the ash (PEARSON, 1970).

Nitrate was determined on a 1% w/v solution of the chelate using brucine reagent (PEARSON, 1970).

Specific rotation was determined on a 1% w/v solution of the chelate using sucrose as standard.

Osmotic pressure was determined on a 10% w/v solution of the chelate using an *Advanced Clinical Osmometer*, Model 3D. From this result, average molecular weight of each chelate was calculated (CHARLEY *et al.*, 1963b).

Infrared analyses were carried out on *Nujol* mull suspensions in a *Unicam S.P. 200* infrared spectrophotometer.

All chelates were chemically analysed to facilitate the prediction of their structure since we have, in the past, not entirely agreed with the structure proposed by other workers. Selected physical properties of each chelate were also determined.

2. Results and discussion

2.1. Concentrations of ferric iron and carbohydrate for chelation

Iron numbers are obviously entirely arbitrary and provide only a means of comparing the relative chelating abilities of each carbohydrate. The iron numbers for the carbohydrates are shown in Table 1 in descending order of the carbohydrates' chelating ability.

Table 1

Iron numbers of carbohydrates

Carbohydrate	Iron number
Fructose	46
High fructose glucose syrup	44
Maltitol	43
Sorbitol	42
Xylitol	42
Hydrogenated 65 D.E. glucose syrup	41
Hydrogenated 43 D.E. glucose syrup	40
Hydrogenated 21 D.E. glucose syrup	39
Xylose	31
Maltose	29
Sucrose	26
Glucose	23
65 D.E. glucose syrup	21
43 D.E. glucose syrup	20
21 D.E. glucose syrup	20

It can be seen that hydrogenation of a carbohydrate in every case increased its ability to chelate iron. This implies that the sugar alcohols are better chelating agents than their unmodified counterparts presumably as a result of the ring structure of the carbohydrate being broken and the sugar being present as the open chain form rather than a mixture of both (infra-red analysis of the isolated chelates as reported later in this study confirm this), the open chain form being apparently more structurally favourable for chelation. In the case of disaccharides and higher saccharides (as in glucose syrups) this implies that only the terminal glucose residue is involved. The structure of fructose (in the open chain configuration) is not unlike that of sorbitol or xylitol (for example 2 CH_2OH groups) and it may be that this structure confers a greater capacity for iron chelation than any other. A similar study has

been reported (DAVIS & DELLER, 1966) using mono- and disaccharides where fructose was found to be the best chelating agent and glucose, lactose, sucrose and maltose were very poor in this respect. This present study confirms these results although the position of hydrogenated carbohydrates in the table suggests that open chain carbohydrates possess some advantages during chelation. It is also shown that chelating ability increases with increase in *Dextrose Equivalent* or D.E. (D.E. is a term used to describe glucose syrups; it is a measure of the syrups' total reducing power towards *Fehling*'s solution, expressed as dextrose, but bears no relation to the actual dextrose content of the syrups. Starch is designated 0 D.E. and glucose, the product of total hydrolysis of starch, 100 D.E. The intermediate values represent various stages in the starch hydrolysis.) This could be anticipated since the proposed structure of the chelate involves the open chain configuration of the carbohydrate and this configuration can only be formed from the reducing end group of an oligomer. The number of such reducing units increases with increase in D.E.

The apparent complexing ability of particular sugars can be influenced by contaminating aldonic acids present by oxidation of the aldehyde group on C-1. Small amounts of gluconic acid for example can achieve solubilization of iron (BATES *et al.*, 1973). It is debatable whether the small amount of acids present could have contributed to the results shown in this study. Thus no purification steps were carried out although it may be necessary in more detailed investigation.

2.2. Preparation, isolation and properties of ferric/carbohydrate complexes

A major noticeable difference between isolated chelates was their colour. This varied from carbohydrate to carbohydrate as shown in Table 2. Table 2 also gives the minimum estimated iron content of each chelate, calculated from the volume of molar ferric nitrate added to the carbohydrate solution. This figure assumes all the iron complexes with all the carbohydrate present, which in practice is unlikely to be the case, and some colloid formation may possibly have occurred. The addition of alcohol to individual solutions of each carbohydrate and iron salt ensured no precipitation unless a chelate had formed. In no case did any precipitate form until the iron and carbohydrate were mixed prior to the addition of alcohol.

2.3. Identification of sugars in the chelates

The interconversion of glucose and fructose in alkaline solutions has been described (KEARSLEY, 1977) and since the chelates are generated in alkaline solution it was considered necessary to establish no interconversion of carbohydrates in the chelates. Paper chromatograms were run using the

Table 2

Colour of chelates and minimum expected iron content

Carbohydrate	Colour of chelate	ml $Fe(NO_3)_3$ added before precipitation of $Fe(OH)_3$	Estimated ferric iron in chelate (% w/v Fe/sugar)
Glucose	dark brown	25.0	11.2
Sorbitol	pale yellow	14.5	6.5
65 B	yellow/brown	8.8	3.9
65 A	pale yellow	12.4	5.5
43 B	yellow/brown	8.2	3.7
43 A	yellow	10.9	4.9
21 B	dark brown	12.6	5.6
21 A	light brown	17.8	8.0
Maltose	yellow/brown	6.9	3.1
Maltitol	yellow	25.0	11.2
Xylose	brown	25.0	11.2
Xylitol	yellow	6.1	2.7
Sucrose	dark brown	7.3	3.3
Lactose	light brown	10.0	4.5
Raffinose	light brown	3.7	1.7
HFGS	khaki	25.3	11.3
Fructose	khaki	25.0	11.2

B = glucose syrup before hydrogenation
A = glucose syrup after hydrogenation

chelated sugars and their standard parent counterparts. In the case of glucose syrups it would be possible that only the glucose and maltose moieties would chelate the iron whilst the higher oligomers played no part, and thus qualitative identification of higher saccharides was carried out using the paper chromatograms. No interconversion was apparent in any case (although quantitative analysis would be necessary to conclusively establish this) and the glucose syrups contained glucose oligomers up to at least maltoheptaose (DP_7). This agrees with a previous report on chelation where no interconversion of fructose and glucose was found (BARKER *et al.*, 1974).

2.4. Analysis and properties of chelates

The results of all analyses are presented in Table 3 in descending order of the percentage of iron in the chelate. The chelates were almost identical in taste; all were bland to slightly salty and slight variations in degree of caramelization were noticeable in some cases. No metallic taints or sweet tastes were noticeable.

Table 3

Analysis of chelates

Carbohydrate	Iron (%)	Na (%)	O.P. (mOsm)	M.W.	Nitrate (%)	S.R. $[\alpha]_{20}^{D}$	Moisture (%)	Ash (%)
Glucose	23.9	3.8	381	526	5.0	23.1	3.5	41.1
Xylose	23.9	4.7	395	506	3.4	—2.7	4.8	42.5
Sucrose	21.0	2.3	247	810	4.8	37.2	2.3	33.0
Xylitol	20.3	3.8	312	641	1.1	—2.2	3.5	36.7
Sorbitol	17.9	4.2	298	672	1.4	14.7	3.5	32.6
HFGS	17.3	5.2	358	558	4.8	—43.2	4.6	32.7
65 B	16.6	2.2	215	930	2.3	92.6	2.5	27.3
Maltitol	14.8	2.3	205	976	2.6	101.7	3.1	26.5
Maltose	14.8	1.9	208	962	1.5	99.4	3.6	24.0
Fructose	14.5	5.0	393	510	3.5	—40.3	4.4	31.8
43 B	13.7	1.3	150	1334	1.7	134.3	2.7	20.7
65 A	13.6	2.8	194	1032	0.6	84.4	3.2	23.8
Raffinose	13.3	1.8	202	990	1.9	95.8	3.9	20.2
Lactose	12.6	2.2	305	656	3.0	22.9	3.2	20.4
43 A	10.6	1.6	123	1626	0.2	121.4	2.9	18.1
21 B	10.1	1.2	134	1492	2.8	154.6	3.2	16.7
21 A	8.4	1.6	155	1290	3.4	149.2	3.3	14.8

O.P. — osmotic pressure
M.W. — average molecular weight
S.R. — specific rotation
B — glucose syrup before hydrogenation
A — glucose syrup after hydrogenation

The chelates were all completely water soluble up to at least 20% w/v and dissolved quickly and easily to give dark, almost black solutions. In a previous report on the analysis of ferric/fructose chelates (using ferric chloride), chloride was reported in the analysis (CHARLEY *et al.*, 1963b) (as we here report nitrate) yet it appeared to play no part in the chelate from the proposed structure, being present perhaps as an impurity. This could explain the variable results for nitrate analysis in this study where no pattern is established amongst the carbohydrates under test. From the data for molecular weight, and sodium and iron analyses, we can approximately determine the molecular formula of each chelate. The mono-, di- and tri-saccharides combined with iron, sodium and water in the ratio $Na \cdot Fe_2 \cdot carbohydrate_2 \cdot x\,H_2O$ (varying amounts of water depending on the carbohydrate). This corresponds almost exactly with the formula proposed by other workers (CHARLEY *et al.*, 1963b), although the spatial configuration of the chelate may still be in dispute. Many structures have been proposed for the chelate, with the iron linked to

the hydroxyl groups of different carbon atoms on the sugar molecule as shown in the following examples: Fe C1,C2 (CHARLEY *et al.*, 1963b); Fe C1,C3 (DAVIS & DELLER, 1966); Fe C1,C2,C3 (BARKER *et al.*, 1974); Fe C2,C3,C4 (KIEBOOM *et al.*, 1977). Obviously, if these authors are correct a great number of possible structures may be formed, and the particular chelate's structure in each case may depend on the conditions of production and isolation. It is difficult to predict any formulae for the glucose syrups since such a wide carbohydrate spectrum is found in these compounds, although basing the calculation on average molecular weight determinations the general formula was found to apply.

The specific rotation of each chelate was also determined. If ferric carbohydrate chelates are compared before and after hydrogenation little difference is seen compared with the results for the carbohydrates alone before and after hydrogenation.

Infra-red spectra were produced for ferric/glucose, /sorbitol, /xylose, and /xylitol. When the spectrum for D-glucose was compared with that for ferric glucose it was seen in the latter case that the ring structure was no longer present and that the ferric glucose and ferric sorbitol spectra were almost identical, showing the carbohydrate in the chelate to be in the open chain form. These spectra are shown in Fig. 1. Similarly, the spectra for ferric xylose and xylitol were almost identical, confirming these findings. With ferric maltose and ferric maltitol the ring structure was intact in both although a reduced signal was found, showing the presence of the intact second glucose residue (NEELY, 1957).

It can be seen by reference to Tables 1 and 3 that the order of the carbohydrates, in relation to iron chelating ability and iron content, is different (except for 21 D.E. glucose syrup) and we conclude from this that whilst one carbohydrate may possess a higher chelating ability than another in aqueous solution, this is not reflected in the percentage of iron in the isolated chelate. We cannot at present offer any explanation for this phenomenon. No yields of chelate were measured during the present study but it was obvious during the work that yields did vary from carbohydrate to carbohydrate, as would be expected. No pattern is shown in Table 3 to relate the structure of the carbohydrate to the iron content of its chelate, which indicates that it is not possible to predict (except on a broad basis) which iron complexes would contain the greatest percentage of iron.

2.5. Effect of carbohydrate concentration on iron chelation

Reproducibility of the chelates' composition is very important especially if such compounds are to be used in clinical applications. The effect of initial concentration of carbohydrate in relation to iron content of the isolated

chelate was thus investigated using glucose and fructose. Previously, 25 ml of a 50% w/v solution of carbohydrate was used and the work was thus repeated using 25 ml of 40%, 30% and 20% w/v carbohydrate solutions, respectively. The chelates were produced as described earlier in this study and the analyses of

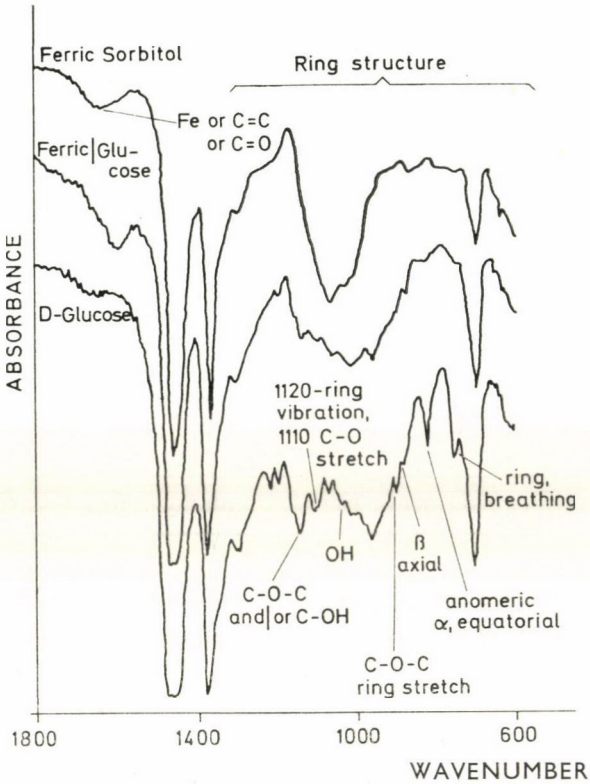

Fig. 1. Infra-red spectra of D-glucose, ferric/glucose and ferric/sorbitol

the isolated chelates are given in Table 4. Two analyses using 25 ml of 50% w/v carbohydrates are also given for reference purposes.

As the carbohydrate concentration decreases the percentage of iron in the isolated chelate increases, presumably owing to the relative concentrations of iron increasing compared with the carbohydrate and the molecular weight of the chelate also increases. An overall fall in moisture content was found. Thus the initial concentration of carbohydrate governs the iron: carbohydrate ratio in the chelate and changes in this concentration will be reflected in the percentage of iron in the chelate. The analyses of the 50% solutions show that at a given carbohydrate concentration, the chelates' composition is reproducible.

Table 4

Effect of carbohydrate concentration on chelate composition

Carbohydrate solution	Iron (%)	Na (%)	O.P. (mOsm)	M.W.	Nitrate (%)	S.R. $[\alpha]_{20}^{D}$	Moisture (%)	Ash (%)	Iron as % ash
50% glucose	23.15	3.6	346	578	4.9	23.1	3.70	38.17	60.6
50% glucose	23.99	3.8	381	526	5.0	23.1	3.46	41.10	58.4
40% glucose	28.44	3.5	304	658	2.6	16.6	1.47	43.81	64.9
30% glucose	28.95	3.2	271	738	2.6	15.8	1.97	46.23	62.6
20% glucose	30.92	4.9	—	—	0.7	—	2.23	49.11	63.0
50% fructose	14.15	5.0	445	450	2.4	−40.6	4.62	30.81	45.9
50% fructose	14.54	5.0	393	510	3.5	−40.3	4.35	31.82	45.7
40% fructose	16.89	4.5	390	512	4.2	−49.8	1.41	33.13	50.99
30% fructose	21.08	4.1	307	652	3.2	−49.7	1.68	39.00	54.05
20% fructose	22.99	4.2	290	690	3.6	−46.9	1.44	39.95	57.55

M.W. — average molecular weight
O.P. — osmotic pressure
S.R. — specific rotation

3. Conclusions

Ferric iron chelates can be formed with a wide variety of carbohydrate compounds. The extent to which the complex forms, *i.e.* chelating ability, is different for each carbohydrate and there appears to be no apparent reason why ferric iron chelates cannot be prepared to some extent with any carbohydrate material, although concentration of carbohydrate may be a limiting factor (*i.e.* limited solubility). The techniques used throughout this present study have been made deliberately unsophisticated to enable rapid "sorting" of carbohydrates and rapid production and isolation of chelates. They nevertheless show interesting differences between carbohydrates, not previously reported and these differences could well warrant investigation of a more detailed nature.

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COMPARATIVE EVALUATION OF PROTEINS OF PLANT AND ANIMAL ORIGIN IN CERTAIN PRODUCTS OF THE FOOD INDUSTRY

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Experiments carried out at the Central Food Research Institute indicate that the necessity of increasing the protein content of animal origin in human diet does not involve the increase of meat consumption. Only the muscular protein is of complete value. The protein content of the connective tissue, due to its collagen and elastine content, is hardly digestible. At the same time it is substantially poorer in certain essential amino acids than wheat which is considered as a very poor source of protein.

In the course of our experiments we supplemented protein of animal origin with that of plant origin in certain food products. For instance we prepared noodles with soy-flour instead of egg and *Hamburger*-type chopped meat using again soy-flour to replace 20% of the meat.

Minced meat samples of different connective and muscular tissue content were mixed with 2% soy isolate and 0.5% blood plasma. The amino acid content and the "in vitro" digestibility was found to be nearly identical.

It was found that for meats of high muscular and low connective tissue content supplementation with plant protein is of interest only from the point of view of improvement in consistency and reduction of costs, while for meats of high connective and adipose tissue content the nutritive value of the meat is increased as well.

THE EFFECT OF TITANIUM UPON THE PROTEIN CONTENT OF VARIOUS PLANT SPECIES

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In the experiments carried out at the Department of Chemistry of the University of Horticulture titanium has proved to be a catalyser of rather universal effect: apart from stimulating photosynthesis it plays an important role in nitrogen metabolism.

The effect of titanium upon the synthesis of protein was first studied in 1976. In different crops (wheat, corn and alfalfa) not only the yield increased by 5–15%, but a 10–15% increase was observed in their protein content, too. Furthermore, in wheat the ratio of basic amino acids improved.

In the small plot experiments carried out with alfalfa during 1977 the yield increased by 5–6%, the protein content by 10–15% and the carotene content by 40–50%. These increases were found significant by mathematical statistical methods. Likewise, the activity of catalase-enzyme in the leaves of plants treated with titanium solution in foliar application has appreciably grown.

In the same year, 1977, a small plot experiment of foliar spray-fertilization of wheat was carried out in cooperation with experts from the Cereals Cultivation Research Institute. In these experiments the yield increased but slightly, by 3–5%, while the gluten content of the flour milled from the grain increased by 6—7% as a result of treatment with titanium. The experiments designed to support the advantageous results have proved that spray fertilization with titanium of 1–15 ppm concentration significantly increased the nitrate reductase activity in wheat and corn plants.

From the viewpoints of both environmental protection and economy it is worth noting that the results were reached with 3 g Ti per hectare and that titanium is not a toxic element.

PREPARATION OF PROTEIN ISOLATES AND CONCENTRATES FROM EXTRACTED OIL-SEED MEALS AND PROPERTIES OF THE PRODUCTS

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Making use of related literature, the authors succeded in developing a method to produce an isolate of 92–94% protein from extracted sunflower seed meal of 43% protein content. Similarly, a method was developed for processing extracted rape-seed meal of 40% protein content.

In developing the methods stress was laid upon keeping the by-product at a feeding value similar to that of lucerne (protein content of 16–20%) after the preparation of the isolate or concentrate. Thus, no waste is produced in the course of the process.

The essence of the process is the extraction of protein from the meals by diluted alkali solution and precipitating the extract at the iso-electric point with acid.

In order to establish optimum conditions for the technology the role of temperature, pH and different solvents was tested. The application of temperatures higher than ambient did not seem necessary. In extracting protein from sunflower seed meal the use of NaOH and for rape-seed meal the use of NaOH with 2% hexametaphosphate at 10.5 pH were found optimal. For precipitation HCl at a pH of 4.5 was optimum for sunflower seed meal and pH 2.5 for rape-seed meal. In order to prevent darkening by chlorogenic acid, 0.25% Na_2SO_3 was added during extraction.

The composition and nutrition biological value of the products were established. The "in vitro", digestibility of the sunflower seed protein was found to be 89. 1% the digestible crude protein was 79.4%, the *Tetrahymena* RNV value was 38%, the *Mitchell-Block* index 63.3%, the *Oser* number 70.8%, the *Bigwood* value 32.1% and the set of essential amino acids was complete with the exception of lysine.

The *Mitchell-Block* index of the rape-seed protein was 80.6%, the *Oser* number 79.2%, the *Bigwood* value 34.6%. As regards essential amino acids, there is a shortage in those containing sulphur. The final products were free of fibre. The amount of health damaging ITC and VTO factors inhibiting nutrient metabolism was found to be below the permissible 0.3% level in the end-product. These data show the products to be suitabe for both human consumption and livestock feeding.

The darkening caused by chlorogenic acid and the attachment of various colorants to protein fractions were also studied by the authors. They found that every protein fraction contained colouring substance, their colour, how-

ever, depends on the pH of the solution. The salt-soluble fraction of the sunflower seed isolate chemically binds the colouring substance. The salt and alkali-soluble fraction of the rape-seed isolate contains also chemically bound colouring substance. These data show the products to be suitable for both human compsumption and livestock feeding.

The technical and economic characteristics of the techniques were checked in pilot plant and industrial scale experiments and were found to be feasible in industrial scale production. The assessment of requirements for the isolates and concentrates in agriculture and industry is currently under way.

STUDY ON REVERSIBILITY OF ENZYMATIC PROTEOLYSIS

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The reverse of enzymatic hydrolysis of proteins is the so-called plastein reaction. This is an enzymatic process resulting in the growth of the peptide chain. The intricate process of plastein reaction includes condensation as well as transpeptidation.

Soy protein hydrolysate was used as substrate. The protein hydrolysate of appropriate molecular weight was obtained by two-fold hydrolyses. First, pepsin was used at pH 1.6, then papain at pH 6 and hydrolysis was continued for 24 h at 37 °C under continuous agitation. The freeze dried hydrolysate was used at a concentration of 30–50% related to the dry matter content. Successful plastein reaction was carried out at pH 6 with papain or at pH 5 with pepsin at a temperature of 37 °C for 48 and 72 h, resp., without agitation.

In the course of the plastein reaction the insoluble product precipitated and the reaction mixture turned into gel.

The reaction was characterized by the productivity percentage:

$$\alpha_p = \frac{10\%\ TCA\ insoluble\ N}{total\ N\ in\ substrate} \cdot 100$$

The grade of substrate hydrolysis was marked

$$\alpha_H = \frac{N\ soluble\ 10\%\ TCA}{total\ substrate\ N} \cdot 100$$

Plastein productivity

Protein	Substrate, α_H	Conc., %	α_p
Soy	84	50	52
Soy	84	50	53
Soy	81	47	58
Soy	81	47	57
Control (without enzyme)	84	50	15

The growth of the chain in the course of plastein reaction was controlled by gel filtration tests, as well.

CHANGES IN THE PROTEIN COMPONENTS OF MIXED FEEDS IN THE COURSE OF GRANULATION

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Since granulation is an energy-consuming process involving high costs, a point has to be sought for where optimum quality of the final product meets production expenses.

This study was aimed at the investigation of physical-chemical phenomena occurring during granulation. Samples prepared according to twenty-six formulae and taken at different phases of production were analysed. Among others, changes in the protein complex were investigated.

Granulated feed samples prepared by pressing and cooling, were compared to those conditioned for a short period with water or steam and having a relatively large grain size range.

The analyses of the protein complex led to the following conclusions:

– a slight decrease in the water-soluble protein content shows that during granulation denaturation of a certain extent occurs;

– the enzyme activity possibly affected by heat treatment during conditioning did not result in the formation of further soluble proteins;

– the change occurring in the quantity and proportions of the soluble fractions indicates a structural change in the proteins;

– as an effect of hydrothermic treatment the colour substance binding ability of the proteins in the samples studied increases; changes are unidirectional. This fact proves that changes occur in the protein structure under the effect of granulation;

– the change – increase – of the amino nitrogen as calculated from the measurement of carboxyl groups is in accord with theoretical considerations. This phenomenon proves the increase in the reactivity of individual groups. It seems probable that due to a degradation of the peptide chain new carboxyl groups will also be formed in some cases. The de-amination of amidized carboxyl groups may involve similar effect.

FORMATION OF AMINO ACID DERIVATIVES DURING THE ALKALINE TREATMENT OF FOOD PROTEINS

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In manufacturing new types of protein, in the course of solubilization' purification, concentration and texturing processes, treatment with alkaline solutions of high temperature is frequently required. According to related literature, during this treatment certain amino acids (cystine, threonine, serine) lose their –SS– or –OH group by beta-elimination. The dehydroalanine thus formed is capable of binding the amino acids of proteins containing basic or disulfide group. The derivative formed with lysine, lysinoalanine, in low concentration (below 100 ppm) and in free state exercises toxic effect on the kidney of rats.

The authors studied the effects of a treatment with 0.1 *N* NaOH at 100 °C for an hour on the following proteins: casein, enzyme-hydrolysed milk protein, ovalbumin, egg protein, gelatine, wheat gluten, soya protein.

Under the given experimental conditions about 30–40% of lysine, serine and threonine was decomposed. The extent of degradation seemed to be independent of the ratio of amino acids in the protein.

As an effect of alkali, a part of the arginine was transformed into ornithine. Its degradation was hardly affected by the mole ratio.

In the proteins studied the amount of cystine left after treatment, showed a close correlation ($r = 0.97$) with the reciprocal of its initial mole ratio. Out of the food proteins examined the cystine present in wheat gluten, egg protein and soya protein was most damaged by treatment with alkali.

The amount of lysinoalanine formed in the different proteins was in direct proportion to the product sum of the mole ratios of lysine, cystine, serine and threonine ($r = 0.98$). Thus it may be concluded that the proteins in milk, eggs and soya are particularly prone to the formation of lysinoalanine.

The authors examined the lysinoalanine content in foods exposed to traditional heat treatment. The lysinoalanine content of some milk protein containing products, such as baby foods, was found to be relatively high (105–220 mg per 100 g protein). In general, with increasing heat treatment the amount of lysinoalanine also increased.

PRODUCTION AND EVALUATION OF PROTEIN OBTAINED FROM *SACCHAROMYCES CEREVISIAE*

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One of the substrates used in plastein production is the protein concentrate obtained from bakers' yeast. Therefore an assay was made to obtain protein from *Saccharomyces cerevisiae* and to establish the quality characteristics of the protein concentrate thus obtained.

Since the aim was the production of reproducible cell-specific protein concentrate, the following efficient methods were compared: disintegration of cells by ultrasonics, with X-press, French press and the digestive enzyme of the snail. Disintegration of the cells was studied by microscopic observation, the extraction of protein by biuret reaction. The protein obtained was fractionated by poly-acryl-amide gel electrophoresis. The optimum conditions of cell disintegration by ultrasonics, using a *Braun-Sonic* apparatus (of 400W capacity), were: yeast concentration 0.05–0.1 g ml^{-1} dry yeast, maximum disintegration at 350 W during a 15 min period.

The results have shown disintegration with X-press to be the poorest method, yielding 10% less protein than disintegration with *Braun-Sonic* apparatus. The yields of disintegration with French press, X-press and snail enzyme differed insignificantly. The gel electrophoretic spectrum of the proteins obtained by the different methods did not show significant difference.

Studying the efficiency of protein extraction it was found that at pH 12, 65% of the extracted protein went into solution. Extension of the extraction period did not increase the protein yield, in subsequent experiments 1 h extraction period was, therefore, applied. With increasing temperature the protein yield decreased.

The protein extracted under the most advantageous conditions at pH 10 and 12 was precipitated at its iso-electric point, freeze-dried and tested for its amino acid composition and nucleic acid content. The nucleic acid content was significantly higher in proteins extracted at pH 10. The gel electrophoretic spectra of proteins extracted at the two different pH values were substantially

similar. The difference in the essential amino acid content of the two proteins was also significant.

Since the essential amino acid content of the protein concentrate is high, after appropriate defatting and deodorization, it may be used in the plastein reaction.

POSSIBILITIES OF THE DETERMINATION OF PROTEIN IN FOODS SPECTROPHOTOMETRICALLY IN THE UV RANGE

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Methods were developed for the determination of protein in products of the meat and dairy industries, based on the measurement of absorption maxima of protein solutions in the 280–210 nm range.

Of the meat products, the conditions of determination were established for salami, Italian salami and saveloy. The samples were minced, homogenized and an aliquot part was suspended in sodium hydroxide solution at 40 °C. A known amount of the suspension was treated with acetic acid and chloroform. After removing the undissolved particles by filtration the absorbance of the solution was measured at the wavelength of the absorption maximum. The optical density value was compared to the percentual protein content as determined according to *Kjeldahl*.

Of the dairy products the conditions of determination were established for cheese varieties and fermented milk products. Cheese was grated, but not homogenized. The clotted milk was homogenized by mixing. The samples were then suspended in a sodium hydroxide solution heated to 45 °C. An aliquot of the suspension was dissolved in acetic acid. In order to prevent the separation of fat chloroform was added only to the cheese samples. The optical density values obtained at the absorption maxima of the solution were compared to the percentage protein values as obtained by *Kjeldahl*'s method.

The accuracy of the protein determination method by photometry is equal to or higher than that of the *Kjeldahl* method according to mathematical tests carried out by the author.

The advantage of this method over that of *Kjeldahl* lies in its simplicity, rapidity and low reagent requirement. It is extremely suitable for on-line control and the control of semi- and final products.

The scheme of analysis is given in the table below:

Operation	Yoghurt	Cheese	Salami	Italian salami	Saveloy
Comminution	—	grating	mincing	mincing	mincing
Homogenization	mixing by hand	—	Biomix	Biomix	Biomix
Suspension	0.1 N NaOH, 45 °C	0.1 N NaOH, 45 °C, mixed by hand	0.1 N NaOH, 40 °C, mixing for 15 | 10 | 5 min in Biomix apparatus		
Dissolution of protein	97% acetic acid	97% acetic acid + chloroform	97% acetic acid chloroform		
Auxiliary operation	—	—	filtration		
Photometry	+	+	+	+	+

CONTRIBUTIONS TO THE PROTEIN DETERMINATION METHOD BY WET ASHING WITH HYDROGEN PEROXIDE ACCORDING TO SARUDI

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Oxidative wet ashing has been applied to biological preparations and foods since 1883 when the total nitrogen determination method of *Kjeldahl* was published. The total mineralization of biological substances and foods of intricate structure is successfully promoted by different oxidizing agents (potassium permanganate, perchloric acid, potassium persulfate, hydrogen peroxide). The accelerating effect of oxidizing agents upon wet ashing increased the interest in these agents too, in addition to intensive and extensive research into catalysts.

The optimal solvent for oxidative disintegration is hydrogen peroxide which was introduced for this purpose by KLEEMANN in 1921. SARUDI improved the accuracy and reliability of the method and proposed it for total nitrogen determination in foods and biological preparations in 1941. The essence of the method according to SARUDI is: the sample to be analysed is predamped with concentrated sulfuric acid, is heated till black and then the hydrogen peroxide is added. The time requirement differs with the substance to be analysed, but is 35–40 min on the average. The time requirement of the complete process using the *Parnass-Wagner* apparatus, is 60 min.

The importance of this method was realized only after 1960, though it has been used in the INSTITUTE FOR FOOD CONTROL AND ANALYSIS, Szeged, as official method. Wet ashing with hydrogen peroxide was first used in feed

analysis. Later the firm A/S N. Foss Electric applied it in its *Kjel-Foss-Macro-Automatic* type automatic nitrogen analyzer.

The aim of the present study was to control the accuracy, reproducibility and the applicability of the method in comparison with the *Kjeldahl* method applying cupric sulfate. In the course of this study the raw protein content of freeze-dried pork (musculus longissimus dorsi), of beef (musculus longissimus dorsi) and of various sausages and a canned meat product was determined, applying hydrogen peroxide. Parallel determinations were made with the same number of samples (20 elements per sample) by the *Kjeldahl* method.

The results of investigations demonstrate that significant difference does not exist between the two methods.

It was found that even with very high protein content (70%) treatment with hydrogen peroxide did not cause a loss of protein. It was found in addition that variations in the protein or fat content from the lowest to the highest did not influence oxidative disintegration.

Summing up the results, wet ashing with hydrogen peroxide as modified by Sarudi is suitable for a relatively rapid analysis with high accuracy of raw materials and products of the meat industry of high protein content.

A RAPID METHOD FOR THE DETERMINATION OF THE QUALITY OF MILK AND SOYA PROTEINS AND THE POSSIBILITY OF DETERMINING THEIR QUANTITY

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The expected increase in the utilization of milk and soya protein for human consumption necessitates their analytical determination. A method earlier developed for the determination of protein in meat products (*Acta Alimentaria 6*, 215–226, 1977) was improved along two lines.

The first aim was to reduce the time requirement of the method. This was achieved by modifying the staining method. After separating the protein fractions by polyacryl amide gel electrophoresis, fixing was carried out in the 40 : 5 : 55 mixture of methanol : acetic acid : water, applying it twice for 1 h each time. The fractions were then oxidized in 1% sodium periodate for an hour, this was followed by reduction with 2% potassium bisulfite. On the gels becoming yellowish brown during reduction the protein fractions appear after 5–10 min in a darker brown colour of iodine. This colour is not stable, it disappears after about 10–15 min. By measuring the location of the fractions during this time, their mobility allows them to be identified. The method

is thus suitable for qualitative determination. The fixing and staining takes about 3–3.5 h against 48–72 h in the earlier method.

The second aim of this study was to develop a method for the quantitative determination of the milk and soya protein added to meat products. To achieve this the fractions separated by electrophoresis were subjected to colour intensity measurement with *Chromoscan* MK II (JOYCE-LOEBL, USA) densitometer, subsequent to staining with 0.2% Fast Green. The standard curve was plotted on the basis of caseinate and *Promine-D* added to the meat; they were heat-treated as described under the qualitative test. (Pure caseinate or *Promine-D* solutions are not suitable for use in plotting the standard curve, because their heat denaturation is different in meat.) Electrophoresis was carried out at a higher gel concentration than earlier ($\sim$ 10%). For both proteins, the smallest amount applied to the gel is 60 μg to obtain results within the margin of error ($\pm$15%). The correlation coefficient is >0.95. An amount of 60 μg per gel corresponds to about 0.6% protein of non-meat origin in the meat products, if consideration is given to preparative operations. The upper limit of linearity for caseinate is 250 μg per gel, whereas for soya protein it is 200 μg per gel.

CHARACTERIZATION OF THE MIXTURE OF COW'S MILK AND BUFFALO MILK BY POLYACRYLAMIDE GEL ELECTROPHORESIS

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The aim of this study was to find out whether polyacrylamide gel electrophoresis was suitable to tell cow's milk from buffalo milk and to discover them in their mixtures of different proportions, furthermore to establish the effect of heat treatment at different temperatures upon the protein content of these milks.

Milk of cows and buffaloes and their mixtures of 25, 50 and 75% were analysed. The samples were pasteurized (at 85 °C) or sterilized (at 120 °C). After defatting, the samples were separated into casein and serum proteins and the two groups of protein were characterized separately. The samples were evaluated on the basis of phoretograms and densitograms taken from the former.

The result has shown buffalo milk to be richer in casein than cow's milk. All the analogous protein fractions exceeded that of cow's milk. In addition, buffalo milk contained a rapidly migrating protein fraction not discovered

in cow's milk. This provides a possibility to distinguish the two kinds of milk even in their mixtures.

On comparing serum proteins, the milk of buffaloes was again found richer and while in cow's milk the amount of the fractions of relatively low mobility, in buffalo milk those of higher mobility prevailed.

Upon heat treatment the amount of serum proteins decreased in both kinds of milk. However, the serum proteins in buffalo milk were found to be substantially more sensitive to heat treatment. The amount of casein proteins increased during heat treatment due to the reduction of the protecting colloid system of the casein proteins and complexes were formed with the serum proteins which migrate with the casein proteins and thus appear as casein proteins in electrophoretograms.

CHARACTERIZATION OF SOLUBLE WHEAT PROTEINS BY ISO-ELECTRIC FOCUSING

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Water and salt soluble protein fractions from the flour and whole crushings of wheat samples, true to variety and grown on the same land in the same year, were compared after separation by iso-electric focusing and determination of the iso-electric points of the separated components.

The iso-electric focusing was carried out in a thin-layer polyacrylamide gel in the presence of *Ampholine* in the pH range of 3.5–10 at 4 °C. The iso-electric points were determined from the pH gradient calculated on the basis of pH values directly measured on the gel layer with specific combined micro surface electrode. To check the pH gradient in the gel, protein preparations of known iso-electric points were used as internal standards as reported in the literature.

The protein solutions separated by iso-electric focusing were found to be extremely heterogeneous. The number of evaluable zones in the crude extracts was 20–24 for whole wheats 15–17 for flours. The purified solution of the protein fraction obtained by precipitation with ammonium sulfate of 0.4–1.74 mole concentrations contained 14–15 zones in whole wheats and 9–10 zones in flours. The iso-electric points of the separated components were found to be in the pH range of 4.3–4.75. Based on the comparison of iso-electric points the following may be concluded:

1. The protein spectra obtained by iso-electric focusing of protein fractions gained from different wheat varieties by identical method are similar;

slight differences may be observed as regards the intensity of the zones and the iso-electric points of some micro-heterogeneous zones.

2. As regards the number and intensity of the separated components the spectra obtained by iso-electric focusing of the protein fractions prepared from whole wheats and flours of the same wheat variety showed greater differences. It is characteristic of the samples of whole wheates that the zones above 7.5 pI are much more intense than those of the flours. Furt hermore, characteristic zones appear in the pH range of 5–6, which are not visible at all, or they are of very low intensity for flours. On the other hand, in flour samples, intense zones of iso-electric points of 4.7 and 4.6 are characteristic.

3. Except for durum variety, the presence of an intense zone of 7.25 iso-electric point was characteristic of all the proteins obtained from all other wheat varieties used for bread. The lack of the zone of pI 7.25 is characteristic of the spectrum of the soluble proteins prepared from durum varieties.

IMPROVEMENT OF THE PROTEIN YIELD OF VEGETABLES WITH ENDO-POLYGALACTURONASE ENZYME

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By the degradation of vegetables with endo-polygalacturonase enzyme, vegetable juices, creams and baby foods may be manufactured. Mixed with enzyme-degraded fruits, vegetable-fruit cocktails may be produced.

The analysis of about 40 kinds of vegetable and fruit products has shown that degradation by enzyme action substantially increased their nutritive value, carbohydrate, protein and vitamin contents.

In the present study the changes occurring in the protein content dissolved in the juice of mashes of different carrot varieties, celery, beetroot, paprika and winter squash were investigated as a function of enzyme concentration and incubation period.

In the endo-polygalacturonase treatment of carrots, squash and green paprika the optimum enzyme concentration (using the liquid enzyme preparation produced at the Central Food Research Institute; SPA_{75}^{Na-p} 300 lh^{-1} ml^{-1}) was found to be 1% and the optimum incubation period 1.5 h.

As compared to the control, the highest protein concentration dissolved in the juice (cca 45%) was found in the juice of green paprika and red tomato-shaped paprika. The enzyme treatment of the celery variety *Apia* resulted in about 40%, that of the carrot variety *Danro* in about 40%, that of the

celery variety *Frigga* in about 30% increase of the protein concentration in the juice.

The highest concentration increase achieved with the lowest enzyme concentration (0.1%) was observed with the enzyme treatment of beetroot.

The lowest protein concentrations as compared to that of the control (15%) were obtained with the carrot variety *Vriflakk* and squash variety *Nagydobosi*.

Under laboratory conditions treatment with endo-polygalacturonase enzyme resulted in an about 15–40% increase of protein solubilization. A similar increase was observed in vitamin and carbohydrate concentrations.

A STUDY OF WHEAT PROTEASES

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The state of enzymes in cereals affects their complex baking value, determines the possibility of their application and gives information as to problems of cultivation and improvement.

Apart from the activity of enzymes, it is important to know the mechanism of individual enzymes, their chemical structure and the characteristics of their active groups.

The aim of this study was to develop a rapid and reliable method for the determination of one of the hydrolytic enzyme components of wheat, of the protease enzyme. The *Anson* test was adapted for use in a continuous automatic analyzer (*Contiflo* Typ., Labor MIM).

The optimal pH value of the isolated enzyme was found to be at 3.8 on haemoglobin substrate. The enzyme activity was found to be stable in the range of pH 2.5–4.5.

The heat tolerance of the enzyme was found to be satisfactory.

The authors tried to clarify the character of the active sites of the enzyme by inhibition tests. Inhibiting agents of SH-enzymes were not, OH reagents and EDTA were successful in inhibiting enzyme activity. Thus, the presence of serine and metallo-proteases is probable.

Further purification of the enzyme preparations (*Sephadex* G 100, G 200) by gel and thin-layer chromatography led to the discovery of further enzyme components.

With the aid of ion exchange chromatography or resolution, enzyme protein components of proteolytic character were detected.

CORRELATION BETWEEN THE QUALITY AND THE PROTEOLYTIC CONDITION OF FLOUR

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The physical properties of doughs prepared of wheat flour are mainly determined by gluten proteins. Changes in the latter entail changes in the physical properties of the dough and the quality of the product. Proteases weaken the dough because they split the peptide bonds of gluten proteins.

The protease activity of flours of about 0.5% ash content on N_α-benzoyl-DL-arginine-4-nitroanylide (BAPA) and bacto-haemoglobin substrate was determined. The flours were milled in a laboratory mill. The protease activity of the flour of 36 sound and 4 over-dried wheat samples was measured. Between the protease activities as measured by the two methods, presuming linear regression, medium close correlation was found. (Coefficient of determination $r^2 = 0.471$.)

The correlation between the protease activity and the baking value was tested in the flour of over-dried wheat and of sound wheat of 0.5–0.8% ash content.

A medium close correlation was found between the protease activity on one hand, and the physical properties of the dough or the volume of the test loaf, on the other. The correlations may be described by a parabola of second order. (The determinative index pertinent to the quality index and established on the basis of the farinogram is: $r^2 = 0.348$. The determinative index related to the volume of the test loaf: $r^2 = 0.718$. Number of data pairs: $n = 143$.) In the case of flours of low protease activity, with increasing activity the quality index and the volume of the test loaf increased. With flours of high protease activity, an increasing activity brought about reduction in both quality index and volume of the test loaf.

The correlation between protease activity and gluten quality was found to be loose ($r^2 = 217$; $n = 143$). With increasing protease activity increased the expansivity of gluten.

A linear, medium close correlation was observed between the protease activity and alfa-amylase activity of the flour ($r^2 = 0.468$; $n = 143$). The investigations have shown the existence of flours of low, medium and high enzyme activity.

INVESTIGATIONS INTO CHANGES IN THE PROTEINS OF WHEAT DURING GERMINATION

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Changes in the quantity and quality, in the proportions of individual fractions and in technical properties of wheat proteins during germination, were studied.

Three series of samples in the order of increasingly vigorous germination conditions were analysed. The effects of approximately normal germination conditions were studied with 10 Hungarian wheat varieties while under vigorous germination conditions the variety *Bezostaya* was used. In addition to gross composition and technological properties, the distribution of protein fractions, as specified by Osborne, was established. The protein fractions were separated by electrophoresis. The amount of free amino acids developed during germination was also determined.

Three phases important from the practical aspect were distinguished in the changes during germination:

– In the initial stage of germination the enzyme activity increased and the degradation of the reserve proteins only started. Changes in the protein fractions were slight. The rheological properties of the dough are sensitive indices of the reduction of quality.

– The degradation of the gluten skeleton is characteristic of the second phase. Severe changes occur in the rheological properties of the dough, in the quantity and quality of gluten. The process of protein degradation may be followed up by different separation techniques.

– In the third phase the degradation of reserve proteins is completed, there is no gluten and dough cannot be made. The proportion of protein fractions substantially changes, more than 50% are albumin and globulin. The amount of free amino acids increases by a factor of 20, however, in the proportion of amino acids or in their distribution within the grain there are significant differences.

RAPE SEED OF REDUCED ERUCIC ACID CONTENT AS A NEW RAW MATERIAL FOR THE VEGETABLE OIL INDUSTRY

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In view of the biological disadvantages of the high erucic acid content of rape seed varieties currently cultivated, an effort is made all over the world to improve and cultivate varieties with reduced erucic acid content.

In Hungary, one of the new varieties, *IR-1*, contains only about 6–10% erucic acid as against about 50% erucic acid level of earlier varieties.

In order to be able to establish production and processing parameters, quality and production costs for the new products, the vegetable oil industry contracted for the cultivation of this variety on an area of 4300 ha during the campaign of 1976/77.

The industry processed 4000 tons separately and manufactured 400 tons of bottled oil and used 1300 tons for hardening and production of margarin.

The experiences gained and the conclusions drawn are as follows:

– On farms where the average yield of traditional rape varieties did not exceed 16–18 q/ha, or was below 10 q/ha the average yield of variety *IR-1* reached a similar level, but on farms where the average yield exceeded 20 q/ha it was 15–20% lower.

– The average oil content of the *IR-1* seeds was lower than that of the traditional varieties, 45.0% against 48.1%.

– Due to a lower oil content the average yield of the oil processing was 13.5% lower than for traditional varieties. At the same time the seed processing capacity of the plant decreased by about 5%. All these factors resulted in a higher manufacturing cost.

– The refinery equipment (Short Mix) was capable of operating without any difficulty or change of capacity.

– The sensory properties of the refined oil were of higher quality than those of the traditional oil. Its taste and odour were less characteristic of rape seed oil. In sensory evaluation it reached a high score (8–9 points).

– Hardening was carried out in the usual way, without difficulties, the hydrogen requirement was however, higher than usual.

– These new types of hardened fats generally contain about 10% more saturated fatty acids and, within these, less behenic acid than traditional rape fats of same slipping points. These cannot be considered to be traditional rape fats. Their properties must be judged by their own standard.

– The fats are suitable for margarin production.

– The ITC and VTO content of the new rape seed variety is higher than that of the traditional varieties.

STUDY OF THE ESSENTIAL FATTY ACID CONTENT OF EDIBLE FATS OF PLANT AND ANIMAL ORIGIN

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In various technological processes fats of natural origin suffer damage not only by saturation and oxidation reactions, but by the isomerization processes of double bonds as well.

The enzymatic determination of cis-cis 1.4-pentadiene-structured fatty acids was the subject of this study. The methods of J. *MacGee* and that of the *IUPAC Working Group* were compared and the linoleic acid content of some major fats was determined.

The essence of both methods is the oxidation of the potassium salts of fatty acids in the presence of lipoxidase enzyme and the determination by spectrophotometry of the quantity of the so formed conjugated hydro peroxides. The two methods are different in the preparation of samples. The two saponification techniques were compared and deviations in the results did not differ significantly.

The continuous UV spectra of the samples were studied and absorption maxima were found to be at 237 μm instead of 234 μm as given in the methods. Since this observation was confirmed by the opinion of several other countries, it was accepted by the Fats and Oils Work Group of IUPAC.

By linking the biochemical method to the determination of fatty acid composition by gas-liquid chromatography the quantity of essential fatty acids within the fatty acids of 1,4-pentadiene structure may be calculated.

The results obtained for some fats of importance in Hungary are given in the following table:

Essential fatty acid content of edible fats

Name of the fat	Fatty acid of c,c-1,4-pentadiene, g/100 g	Linoleic acid, g/100 g
Rama margarin	9.6	9.3
Liga margarin	6.1	5.0
Edible fat	0.3	0.3
Butter	3.3	3.3
Rape-seed oil	29.2	22.7
Sunflower-seed oil	69.4	68.3
Soy oil	63.0	55.6

NEW RESULTS OBTAINED BY THE SPIN-LABEL EPR-SPECTROSCOPIC TECHNIQUE DEVELOPED IN HUNGARY TO STUDY THE PHYSICAL STRUCTURE OF BUTTERFAT

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Research in recent years has sufficiently revealed the fat structure created in cream and in water-free milkfat by temperature gradient cream ripening ("winter" and "summer"). NMR measurements gave quantitative

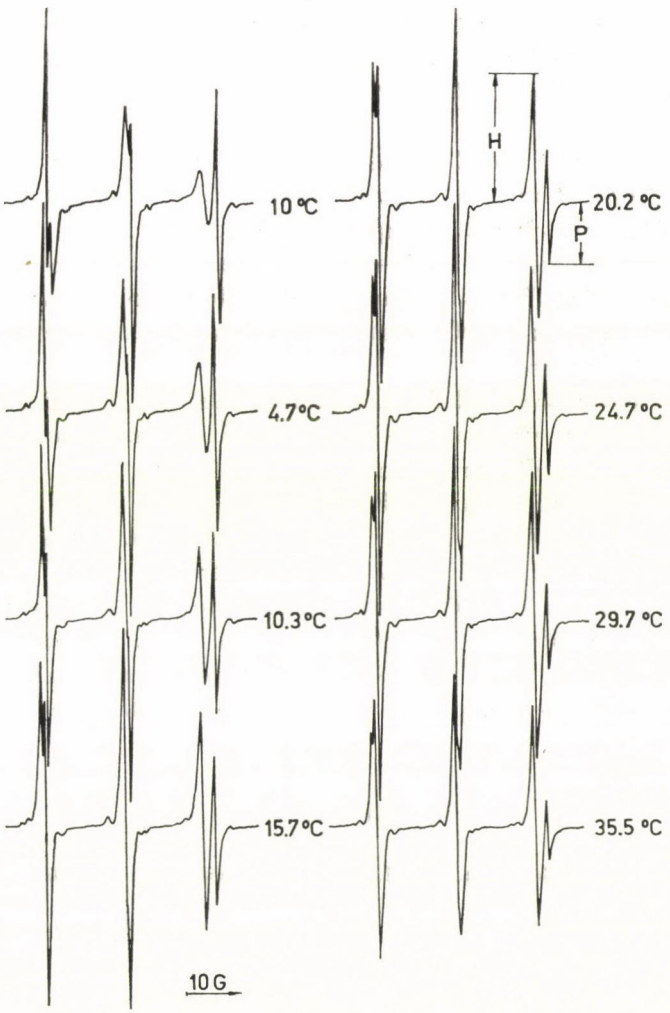

Fig. 1

information on winter ripening (*Alnarp* method), resulting in 8–10% more fluid butter oil in the fat phase of cream in contrast to summer ripening of the same cream. Since similar data in relation to butter are not available, the aim of this study was to develop a method of measurement which provides information on the butter oil content of the fat phase of butter prepared by various methods of ripening.

In the course of the analysis of different nitroxide radicals a labelling substance was found in the spectrum of which the index of water may be separated from that of the oil phase. This substance is 4-oxo-2,2,6,6-tetramet hyl-piperidin-1-oxyl.

The butter to be tested was produced under experimental conditions. The labelling substance was added in the final phase of kneading at a proportion of 0.5 μl 10^{-2} mole per 1 litre to 100 mg butter. The labelled substance was kept in a cuvette for 24 h at 4 °C. Measurements were carried out in the temperature range of 0–40 °C at 1 °C intervals on a *ER-9* type *EPR* spectroscope.

Figure 1 illustrates the EPR spectra of butter at different temperatures. Parameters H and P are also shown. The separation of the third peak into two marks (oil and water phase) is apparent. As it may be seen, with increasing temperature the concentration of radicals in the oil phase increases.

To characterize crystallization the $f = H/P$ quotient was introduced according to SHIMSHICK & MCCONNELL (1973). This is shown in Fig. 2 as the function of temperature.

In the butter prepared from cream ripened by the winter technique (6–20–12 temperature gradient) the liquid to solid fat ratio was lower at every temperature than in butter ripened by the summer technique (20–6–12 temperature gradient), as is shown in Fig. 3.

This observation seems to contradict the theory according to which, at identical fatty acid composition, a higher butter oil content participates in the production of a butter of spreadable consistency. It supports, however, the corpuscular-homogenous theory of butter. Thus, a butter of corpuscular structure (winter ripened) is more suitable for spreading than the homogeneous one if, at the given temperature, the liquid–solid fat ratio is lower.

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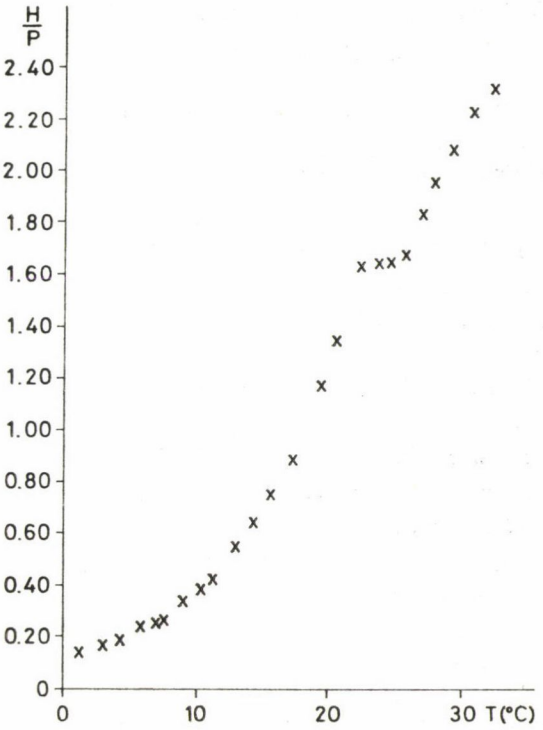
H/P
2.40
2.20
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0
0
10
20
30 T(°C)

Fig. 2

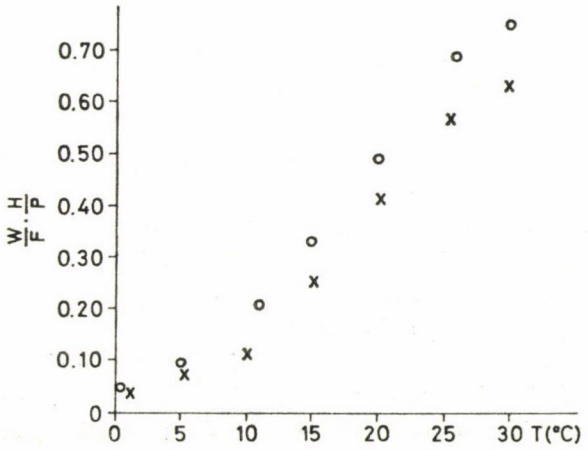
W.H/P
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
0
5
10
15
20
25
30 T(°C)

Fig. 3

INVESTIGATION INTO THE LIPID COMPOSITION OF SOME EDIBLE MUSHROOMS

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The lipids of some edible mushroom species: *Agaricus bisporus, Agaricus arvensis, Agaricus silvaticus, Clitocybe nebularis, Amanita rubescens, Hygrophorus eburneus* and *Polyporus squamosus* were investigated for the distribution of lipid classes. The cultivated mushrooms were provided from trade, the wild growing species were collected in the surroundings of Budapest. The isolation of individual lipid classes was carried out by column and thin-layer chromatographic technics. The lipids extracted with acetone and chloroform/methanol 2 : 1 were fractionated on *Florisil* column by stepwise elution with changing the solvent polarity. The obtained fractions were examined by TLC on *Kieselgel G* layers. Spots were detected with iodine vapours, *Liebermann-Burchard* reagent, ninhydrin spray, *Dragendorff* reagent, orcinol spray, resorcinol spray and *Hanes* reagent, resp. The distribution of the lipid classes of the individual species is different, their further identification needs additional research work. – The fatty acids of the non-polar and polar lipids of *Boletus edulis, Suillus branalatus, Xerocomus chrysenteron, Pleurotus ostreatus, Armillariella mellea, Agaricus arvensis, Agaricus silvaticus* and *Clitocybe nebularis* were separately saponified and converted to methyl esters. The fatty acid composition was determined by GLC.

The occurrence of C_8–C_{24} saturated and unsaturated fatty acids was established. Among the saturated fatty acids palmitic acid is predominant (in order of the above-mentioned species in non-polar lipids 10.98%, 9.22%, 32.03%, 11.46%, 22.67%, 20.07%, 19.29% and 11.72%; in polar lipids 11.95%, 14.08%, 35.39%, 3.23%, 3.84%, 6.08%, 12.65% and 7.88%. Stearic acid is only present in smaller amounts: in non-polar lipids 6.59%, 2.42%, 15.82%, 1.94%, 10.62%, 7.58%, 8.01% and 2.95%, in polar lipids 0.4%, 0.94%, 18.06%, 2.41%, 1.20%, 1.73%, 4.56% and 4.54%. The principal unsaturated acids are linoleic acid (in non-polar lipids 30.47%, 66.00%, 7.03%, 61.78%, 12.17%, 40.09%, 53.18% and 27.92%, in polar lipids 75.19%, 71.88%, 22.98%, 51.87%, 60.27%, 86.19%, 16.99% and 59.99%) and oleic acid (in non-polar lipids 44.78%, 19.01%, 30.71%, 16.63%, 45.09%, 7.04%, 8.32% and 16.88%, in polar lipids 7.95%, 7.30%, 6.13%, 25.23%, 20.45%, 2.97%, 3.45% and 8.59%. The $C_{20:1}$ acid was found to be the principal unsaturated fatty acid only in the nonpolar lipids of *Clitocybe nebularis* and in the polar lipids of *Agaricus silvaticus* (40.53% and 33.64% resp.), and larger amounts of the $C_{16:1}$ acid were found in the non-polar lipids of *Agaricus arvensis* (14.60%) and *Armillariella mellea* (7.20%).

ANALYSIS OF THE ESSENTIAL FATTY ACID CONTENT IN *BORAGINACEAE* SEEDS

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According to preliminary investigations the seeds of plants belonging to the family of *Boraginaceae* might be considered as a raw material for prostaglandin production in view of their 6,9,12-octadecatrienoic acid (gamma-linolenic acid) content.

Sixty-seven samples belonging to 31 species of the family of *Boraginaceae* were analysed. In some cases samples belonging to the same species but grown at different locations were compared. Nearly all the samples were found to contain gamma-linolenic acid. In about 43% of the samples the gamma-linolenic acid content of the oil exceeded 10%.

The analysis by gas chromatography of the fatty acid esters of *Borago officinalis* has shown the oil obtained from the seeds to contain 19% gamma-linolenic acid. For the purpose of quality identification the 6,9,12-octadecatrienoic acid of *Sigma* was used. From the ester mixture of the oil, gamma-linolenic acid of 97% purity was isolated. This was used in the Chinoin Factory for Pharmaceutical and Chemical Products to manufacture tritium-labelled, biologically active prostaglandin.

In the course of evaluating the results a component hitherto unknown was discovered which occurs in the oil obtained from the seed of several plant species belonging to the *Boraginaceae* family. The oil obtained from the seed of *Lappula squarrosa* contained 5.4% gamma-linolenic acid, 40.2% linolenic acid and 16.4% of the unknown component. This component was separated in 90% purity from the ester mixture. Then the firm Jeol in Tokyo identified this component by the GC/MS technique as octadecatetraenoic acid.

This result is important from the theoretical aspect since to date gamma-linolenic acid and octadecatetraenoic acid were found only in the assimilating tissues of moss and fern species within the vegetable kingdom. From practical aspects these plants may serve as a source in the production of fourfold unsaturated fatty acids of 18 carbon atoms.

On analysing the samples, erucic acid was also found in the ester mixtures. In one of the samples 17.8% erucic acid was found and this was then transformed into biologically active feromon.

MUTUAL EFFECT OF LIPIDS AND PROTEINS IN WHEAT PROTEINS

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The composition of the lipopurothionin complex, lipids in interaction with purothionin and free lipids, and the nature of mutual effects between proteins and lipids were investigated.

Purothionin was obtained by four different techniques from the petroleum ether extract of wheat flour: a. by the traditional hydrochloric acid method; b. by precipitating the lipoprotein with methyl acetate and breaking down the isolated complex; c. by breaking down the molecular fractions of the b. complex; d. by separating on *Sephadex* LH-20 the protein-positive fractions. The isolated protein and lipid preparations thus obtained were analysed by different analytical methods. The main conclusions drawn are as follows.

The lipopurothionin isolated with methyl acetate according to *Elton* and co-workers, consists of 3 parts of protein and 7 parts of lipid. Every purothionin fraction could be identified in the apoprotein. The lipid fraction consists mainly of phosphatidyl ethanol amine, phosphatidyl inosit and two glycolipids. In addition it contains sterol as esters.

On comparing the composition of lipoprotein fractions according to molecule the complex may be considered an unstable product of statistical composition in which the protein and lipid components are in interaction of ionic character.

The lipid composition of the protein-positive fractions of the petroleum ether extract differs from the above by containing a substantial amount of (nearly 50%) glycerid.

On the basis of the data obtained it appears that the lipopurothionin obtained by precipitation with methyl acetate is a product formed after the breaking off of components bound to the complex by hydrophobic interaction. Thus, in the original protein-lipid complex as present in wheat, hydrophobic interactions are found between the apolar amino acid side chains and the glycerids (protein-lipid) and between the fatty acid side chains of phospholipids and the glycerids (lipid-lipid) as well as ionic interrelations between the basic side chains of protein and polar lipids.

A STUDY INTO THE PHOSPHOLIPID COMPOSITION OF FOODS

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The separation and quantitative determination of biologically important phospholipids has not yet been resolved satisfactorily. The analysis of foods requires a method of short duration and low material needs and which is equally suitable for serial tests. The aim of this study was to develop a method meeting these requirements.

To separate the phospholipids from neutral fats, column chromatography was applied. Of the adsorbents tried (silicic acid, DEAE cellulose, florizil) silicic acid proved to be the most suitable as far as reproducibility and recovery are concerned. To separate about 100 mg total lipid content 2 g silicic acid was used. The sample was dissolved in chloroform and thus applied to the column. The neutral fats were eluted with chloroform while the phospholipids with methanol.

The phospholipid fraction obtained by column chromatography was separated into components on *Kieselgel* H layer with the 75 : 30 : 4 : 0.5 mixture of chloroform : methanol : ammonium hydroxide : water. To visualize the developed chromatogram phosphoric acid copper acetate was used.

Composition of phospholipids

Sample	Total fat content	Neutral fraction	Phospholipid fraction	Phosphatidyl choline	Phosphatidyl ethanol amine	Sphingomyelin	Phosphatidyl serin + phosphatidyl inositol	Lisophosphatidyl cholin
	%			as % of the total phospholipid cntent				
Egg-yolk	31.7	70.6	29.4	69.6	23.5	5.7	traces	1.2
	±0.5	±0.8	±0.8	±3.3	±2.0	±1.8		±1.1
Cow's milk	2.8	95.3	4.7	36.0	24.8	38.6	traces	—
	±0.2	±0.3	±0.3	±2.5	±1.8	±3.4		
Moderately-fat cottage cheese	7.1	94.7	5.3	32.7	30.3	37.0	traces	—
	±0.8	±1.7	±1.7	±4.6	±2.9	±3.8		

For the quantitative determination of phospholipids the chromatograms were evaluated with a densitometer. The evaluation required the preparation of a separate calibration curve for each phospholipid (phosphatidyl choline, phosphatidyl ethanol amine, phosphatidyl inositol, phosphatidyl serin, sphingomyelin).

The methods described were used to determine total fat content, phospholipid and neutral fractions and composition of phospholipid fraction in egg-yolk, cow's milk and moderately-fat white cheese. The results are given in the table.

Nine samples of different origin from each foodstuff were studied; the table contains average values and related deviations.

DETERMINATION BY GAS CHROMATOGRAPHY OF STEROLS IN FOODS

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In the development of cardiac and vascular diseases hypercholesteremia is one of the important hazard factors according to clinical experience and results in research work. In recent years great effort is therefore made to discover substances of natural origin, which are capable of reducing the cholesterol level of the blood serum. Plant sterols are such substances and their effect on cholesterol has been known since the 'fifties.

Since the composition and the quantity of sterols in foods is little known a method was developed for the qualitative and quantitative determination of these substances.

The following are the main steps of this technique:

1. Extraction of fats from the sample.
2. Saponification of the extracted fat (with potassium hydroxide in methanol or with tetramethyl ammonium hydroxide).
3. Extraction of the unsaponifiable part and determination by gravimetry.
4. Separation of the sterol fraction by preparative thin-layer chromatography (carrier: *Kieselgel* G, running mixture: petrolether : ether : glacial acetic acid 90 : 15 : 2).
5. Elution from the layer and determination of the total sterol content by gravimetry.
6. Determination by gas chromatography of the free sterols.

Conditions of gas chromatography were as follows:

apparatus: *Carlo-Erba Fractovap* 2400 T
packing: 80–100 mesh *Gaschrom* Q moistened with 3% OV 17
column: 9 pyrex glass of 1.3 m length and 0.4 mm inner ∅
carrier gas: nitrogen of 0.9 kg per cm^2 pressure
injector temperature: 290 °C
column temperature: 260 °C

The internal standard applied in quantitative determinations was androsterol. The method is suitable for measuring to 1 μg.

Recovery: 96.5 $\pm$ 3.5%.

The method was used to determine the sterol content and composition of commercially available sunflower seed and rape-seed oils, industrial soy oil, commercial cow's milk and breast milk. Data are given in the table below.

Sample	Total sterol content mg per 100 g fat	Cholesterol	Campesterol	Stigmasterol	β-sitosterol	Brassica sterol	Other
		in percentage of the total sterol content					
Sunflower-seed oil	0.76	—	15.6	10.4	54.1	—	19.9
Rape-seed oil	0.61	—	33.8	—	56.7	9.5	—
Soy oil	0.42	4.5	19.9	17.3	58.3	—	—
Cow's milk with 3.6% fat content	237.9	93.5	2.2	—	—	—	4.3
Cow's milk with 2.8% fat content	237.0	92.8	1.9	—	—	—	5.3
Breast milk with 4.2% fat content	245.4	91.7	—	—	2.1	—	6.2

NATURAL ADDITIVES TO PREVENT RANCIDITY IN FATS

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To inhibit oxidative spoilage of fats natural additives of antioxidant effect were used.

The additives used were ground paprika, seeds of paprika, tomatoes and of other plants appearing as waste material at processing. Paprika and the seeds were ground and added to the fats in this form and also as isolates. The latter were obtained after different experiments to extract the active agent. To control the efficiency of the additives, samples without additive and with added BHT (butyl hydroxi-toluol) were also oxidized.

The oxidization experiments were carried out at 20, 50, 70 and 100 °C, resp., in Petri dishes. The extent of oxidation was established by the method of *Lea* and the final point of titration was controlled with a Dead-stop titrimeter. The results have shown all the additives to be effective antioxidants. Ground paprika was extremely effective, but its use is limited by its colour. The fat mixed with tomato seeds had a peroxide number of 8 after exposure to rancidification for 800 h at 50 °C, while the peroxide number of the control was

186. Even after 8 months at room temperature the fat samples mixed with tomato seeds were better than the sample containing BHT. Tocopherols and other fractions obtained by extraction with solvents were not as effective as the seeds themselves. Many experiments were carried out to extract and identify the active agent. Tocopherol was identified as a primary antioxidant, while citric acid, cholamine, cephalin and lecithin as synergists. On the basis of the experiments the author considers that in addition to the identified primary and secondary antioxidants other compounds in complex binding and adsorption forces play a role in inhibiting rancidity.

The author suggests the utilization of seeds as natural materials, to inhibit the oxidation of feed and fat used as feed. Apart from their antioxidant activity their nutritional value justifies also their use for this purpose. Their oil content is 24–28% and they do not contain erucic acid. They contain 18–22% protein and 21–24% carbohydrate. Out of their biologically active components, tocopherols and phospholipids are not negligible either.

Further experiments are necessary to be able to replace synthetic antioxidants in foods by natural materials.

APPLICATION OF ATOM ABSORPTION SPECTROMETRY IN THE VEGETABLE OIL INDUSTRY

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Metal contamination in fats (Fe, Ni, Pb, *etc.*), in view of its biological disadvantages and its spoilage-accelerating ability, deserves increasing attention all over the world. In recent years a number of metal determination methods have been developed, based mainly on atom absorption photometry.

The authors have started to assess metal contamination in raw materials, semi- and final products of the Hungarian vegetable oil industry. A *Zeiss* AAS_1 type flame ionization atomic absorption spectrophotometer was used. The present paper contains the part of the research work related to the most critical contaminant of hardened fats and margarins, to nickel. It discusses the effect of various methods of preparation, the sensitivity and scatter of direct and indirect atomic absorption technique and the comparison of different methods.

In the course of preparation for the indirect method, the nickel was extracted from the petroleum ether solution with diluted nitric acid and the nickel content of the acid solution was established in comparison to a standard nickel solution made of metal nickel.

Characteristics of the method (λ = 232 nm):
sensitivity: 0.2 ppm Ni/1% absorption
measuring limit: 0.002 ppm Ni.

Deviations belonging to different concentration values based on 5 parallel measurements each:

Serial number of sample	Ni content average (ppm)	Standard deviation (ppm)	Coefficient of variation
1	3.80	0.18	4.74
2	2.40	0.21	8.75
3	0.49	0.022	4.48
4	0.04	0.01	25.00

In the course of direct measurement, the 10% methyl-iso-butyl keton solution of the fat was sprayed in air-acetylene flame. The standard used was rape-seed oil solution of nickel-cyclohexeno-butyrate.

Characteristics of the method (λ = 232 nm):
sensitivity: 0.2 ppm Ni/1% absorption
measuring limit: 0.2 ppm Ni per fat.

Deviations belonging to different concentration values based on 5 parallel measurements each:

Serial number of the sample	Ni content, average (ppm)	Standard deviation (ppm)	Coefficient of variation
1	18.04	1.13	6.26
2	5.37	1.12	20.86
3	5.04	0.90	17.86
4	0.27	0.05	18.52

Of the two methods the direct method is more suitable for on-line measurements.

In one of the oil hardening plants the direct method was used to follow step by step the nickel removing effect. The results have shown the deacidification operation (in the continuous operation *Short Mix* equipment) to remove 80% of the nickel content, on the average. The effect of clarification is also important (0.15% fuller's clay): it reduces the nickel content to a quantity below the measuring limit.

OCCURRENCE OF POLY-AROMATIC HYDROCARBONS IN FATS AND THEIR QUANTITATIVE CHANGES UPON HEATING

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Carcinogenic and cocarcinogenic polyaromatic hydrocarbons (PAH) may contaminate foodstuffs and among them edible oils too. The most effectively carcinogenic representative, 3,4-benzpyrene serves as indicator of contamination with other PAH compounds. Data on the PAH contamination of Hungarian edible oils were, so far, not available. Data in the literature are contradictory as regards the effect of heating upon PAH contamination in oils.

The investigation of this subject led the author to the following conclusions:

Samples of edible oils, margarines and hardened fats contained 3,4-benzpyrene in the range of 1–10 $\mu g\ kg^{-1}$, which is above the permissible limit. This necessitates the revision of the vegetable oil processing technology.

Deep-fat frying tests in the laboratory and samples taken from sunflower seed and rape-seed oil used in commercial fish or dough frying shops have unambiguously proved that heating to 200–300 °C reduces the 3,4-benzpyrene content of oils.

At a relatively lower temperature, at 120 °C the 3,4-benzpyrene content of the oil may increase, if for instance, 3,4-benzpyrene is introduced with potatoes into the system and this is not counterbalanced by the rate of degradation.

However, this does not mean that oils may be used for an unlimited period. Apart from a series of unfavourable changes, the formation of acrolein of toxic and mutagenic effect necessitates the limiting of the use of oil.

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OCCURRENCE OF BACILLUS CEREUS IN FOODS

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(Received February 16, 1978; accepted August 25, 1978)

In the course of investigations into the mechanism of non-specific food poisonings the *Bacillus cereus* content of various foods was studied. Within ten months out of the 2800 food samples analysed none were found responsible for food poisoning. In 100 samples (3.57%) *B. cereus* was detected.

B. cereus was present in 31 samples at a cell count level of 10^5 g^{-1} and in 21 samples this bacterium formed more than one half of the microbial population. The high cell count and high proportion, in themselves, are not sufficient to cause food poisoning. Other factors, hitherto not known, such as microflora, environment and the resistance of the organism attacked, are of significance.

The two main fermentative activities of *B. cereus* are the haemolysis and lecithinase activity. These two characteristics suffice for bacteriological identification. Proteolytic activity is not suitable for the differentiation of this species from other members of genus *Bacillus*. Carbohydrate fermentation without gas formation may be informative of the origin of contamination.

A part of the cases of food poisoning is caused by facultative pathogens or non-specific microorganisms (KUHN, 1964; CSERENYEY, 1975; HOBBS, 1976). The facultative pathogens belong to various genera and their only common property is their effect upon the intestinal tract (SEIDEL, 1958; MOSSEL *et al.*, 1963; GOEPFERT *et al.*, 1972; NIKODÉMUSZ, 1976). The food poisoning effect of aerobic spore-forming rods, belonging to these groups of microorganisms and among them that of *Bacillus cereus* was only relatively recently discovered (BUTTIAUX, 1956; NIKODÉMUSZ, 1958; MUSCHTER & SEIDEL, 1967). Poisoning occurs only if the pathogen is present in the food at the time of consumption in a concentration of at least 10^5 g^{-1} and forms at least 50% of the total cell count (BUTTIAUX, 1956; NIKODÉMUSZ, 1962; KIM & GOEPFERT, 1971; GOEPFERT *et al.*, 1972; HOBBS, 1974). These two preconditions were proved to be essential in all cases of food poisoning, caused by spore-forming bacteria and detected between 1958 and 1962 in Hungary (NIKODÉMUSZ, 1962). However, it is undeniable that, in addition to the sensivity of the organism, other factors are also required for the development of illness. These are partly dependent on the microorganism, partly on the environment (GOEPFERT *et al.*, 1971; GILBERT & TAYLOR, 1975, 1976; NIKODÉMUSZ, 1976; TURNBULL, 1976; GILBERT & PARRY, 1977). In the course of the study on the mechanism of poisoning first the characteristics of *B. cereus* were investigated.

1. Materials and methods

First the quantity and proportion of *B. cereus* in foods, then their fermentative activities were studied. In the years 1966–1967 the samples brought in by the sanitary authorities and collected by the author were investigated for their microbiological parameters at the Institute of Nutrition, Budapest. Foods prepared by institutional catering establishments, confectionary products, meat and milk products from the market and dried foods were analysed. The samples were collected and prepared according to the instructions of *Polónyi* (POLÓNYI, 1954; NIKODÉMUSZ, 1974). The total viable cell count (number of colony-forming units) was determined on agar nutrient containing 20% skimmed milk and the presence of *B. cereus* was also established on these. Colonies suspicious for *B. cereus* (colonies of 5–10 mm diameter, of irregular border line, greyish colour, grainy and easily smearable) were injected onto agar plates containing blood or egg-yolk. The behaviour of *B. cereus* in these two media is typical [on blood agar colonies resembling powdered glass or moiret silk and having a haemolytic zone of 5–10 mm, on egg-yolk agar yellowish-white colonies with 5–10 mm precipitate zone, are formed (egg-yolk reaction); both reactions occur in colonies at the age of 4–6 h] therefore suitable for bacteriological diagnosis, except for a few exceptions (NIKODÉMUSZ, 1976).

2. Results

During a 10-month period it was tried to obtain pure cultures of *B. cereus* from 2800 food samples not causing food poisoning upon consumption. In 100 samples was *B. cereus* identified corresponding to a frequency of 3.57%. The total microbial count and the number of *B. cereus* cells was determined and from these the percentage of *B. cereus* could easily be calculated. Enumeration was carried out on milk agar, since other media suitable for the isolation of *B. cereus* (MOSSEL *et al.*, 1967; KIM & GOEPFERT, 1971) inhibit the growth of other microorganisms and thus give higher proportions of *B. cereus* than the true value. The quantity and proportion of *B. cereus* in the 100 samples was as shown in Tables 1 and 2.

As it may be seen in the tables the number of *B. cereus* exceeds 10^5 g^{-1} in 31 samples and in 21 samples it exceeds 60% of the total viable cell count. None of the foods caused poisoning. It is true that not all cases of poisoning become known to the medical and sanitary authorities, however, it is hardly believable that poisoning propagated by ice cream and by pastry filled with vanilla cream could remain undiscovered as both items are consumed by many people. Of the 100 *B. cereus* infected samples 55 were of these two items

Table 1

Distribution of B. cereus *in different foods according to type of food and cell count*

B. cereus count (g^{-1})*	No. of samples containing *B. cereus*				Total No. of positive samples
	Type of food				
	Pastry filled with cream	Ice cream	Meat products	Other foods	
10^3	13	11	2	17	43
10^3–10^5	11	1	4	10**	26
10^5–10^6	7	2	6	1	16
10^6	7	3	—	5	15
Total	38	17	12	33	100

* The smallest amount detectable is 20 g^{-1}
** In 6 samples *B. subtilis* was found at a cell count level of 10^3 g^{-1}

Table 2

Proportion of B. cereus *in the total viable cell count* (Results of colony enumerations on milk agar)

Proportion of *B. cereus*, %	Number of samples
< 1	31
1 – 10	24
10 – 20	5
20 – 40	9
40 – 60	10
60 – 80	7
80 – 90	7
90 –100	7
Total	100

and in 19 of them cell count exceeded 10^5 g^{-1}. Thus, in this case it must be accepted that poisoning was not caused.

In 94 samples spore-forming bacteria other than *B. cereus* were not found. In 6 samples (ready-made meals), *B. subtilis* was found at the count of 10^3 g^{-1}. Of the spore forming bacteria, *B. subtilis* occurs most frequently in food in Hungary (NIKODÉMUSZ, 1962, 1976). In this case no trouble was caused by its presence.

Further the main biochemical characteristics, the lysis of proteins, carbohydrates and lecithin and sensitivity to penicillin, were studied. According to the results – in agreement with data in the literature (HALMANN, 1974) – all the strains were found to be catalase positive and indol negative, they did not produce hydrogen sulfide, liquified gelatin, but not the coagulated blood serum (*Loeffler*'s medium). They were resistant to penicillin. Other, not homogeneous characteristics are given in Table 3.

Table 3

Main fermentative characteristics of 100 B. cereus *strains*

Type of activity	Number of strains and result of test	
	positive +	negative −
Decomposition of protein and fat		
Haemolysis	99	1
Lecithinase	99	1
Coagulation of milk	100	—
Peptonization of milk	65	35
Fermentation of carbohydrates		
Lactose	8	92
Dextrose	100	—
Saccharose	69	31
Maltose	97	3
Mannit	9	91
Laevulose	99	1
Salicin	72	28
Xylose	8	92
Arabinose	14	86

Of the biochemical tests the haemolysis and lecithinase are the most important because these two are characteristic of the species *B. cereus*. The egg-yolk reaction may be considered specific for *B. cereus*, because the only other aerobic spore forming bacterium reacting in this way is *B. mycoides*. This, however, can easily be differentiated from *B. cereus* due to its root-like colonies. Some *Staphylococcus aureus haemolyticus*, *E. coli haemolyticum* and *Pseudomonas aeruginosa* strains also degrade lecithin, however, they can be separated on the basis of their colonies. Positive reaction is given by *Clostridia*, however, they do not multiply in the presence of free oxygen. Lecithin is not degraded by the so-called R-type *B. cereus* strains which are present in

1–2%. According to the opinion of some authors the lysis of lecithin is a common characteristic of food poisonings, however, this is not yet proven sufficiently (GOEPFERT *et al.*, 1971). Haemolysis is a less specific reaction, it is given also by *B. subtilis* and other *Bacillus* species. *B. subtilis* may be differentiated from *B. cereus* on the basis of their colonies. Haemoglobin is not degraded by the "sotto" variety of *B. cereus*. This variety was described in Japan, however, it may be encountered in Hungarian foods, as well (NIKODÉMUSZ, 1976).

Milk was coagulated by all of the 100 strains. All contained caseolytic enzyme, while only 65 were able to peptonize. Thus, it seems that there are differences in the enzymes.

The Table shows also that almost all the strains degrade fructose and maltose and, as mentioned earlier, glucose, too. The fermentation of these carbohydrates without gas formation is not species-specific, many microbes are capable of fermenting sugars. The acid formation from salicin, however, may be considered typical, while saccharolysis is not characteristic for *B. cereus* only. The majority of the strains did not ferment mannit, xylose and arabinose.

The degradation of sugar is not suitable for the identification of *B. cereus* or for its separation from the other species of the genus *Bacillus*. However, it may serve to establish the origin of *B. cereus* strains. In the case of poisoning the pathogen may get into a food from different raw materials. If several strains are present, that one will be responsible which degrades sugar in the same way as the strain causing poisoning. In such cases the fermentation of carbohydrates complements sero-typing, phage-typing and the results of antibiogram determination. Identical behaviour renders probable, contrary behaviour excludes, identity.

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USE OF ENDO-POLYGALACTURONASE TO INCREASE THE DISSOLVED PROTEIN CONTENT OF VEGETABLE JUICES

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Endo-polygalacturonase (endo-PG) is able to disintegrate the insoluble pectic materials between cells of vegetable tissues and, as a consequence, it increases the stability of the juices. As cell walls remain uninjured, a high recovery of valuable materials, vitamins and nutrients can be attained after enzyme treatment.

In this work the protein content of vegetable juices produced by endo-PG treatment was compared with that of the original tissues and with the vegetable juices produced without enzyme. Protein solubilization of enzyme-treated squash, green pepper, red tomato-shaped paprika, carrot and red beet was investigated as a function of the time of incubation and of the concentration of enzyme, resp.

A concentration of 1% of the liquid enzyme preparation was found best in the case of squash, green pepper and carrot, at an optimum incubation period of 1.5 h. In the case of red tomato-shaped paprika, celery and several varieties of carrots the use of 1.0% enzyme and an incubation period of 3 h proved to be optimal, while in the case of red beet, an enzyme concentration of 0.1% at an incubation period of 1.5 h resulted in the highest protein solubilization.

Protein contents of the endo-PG-treated vegetable juices increased by 14–50%, compared to that of the untreated control. This means that enzyme-treated vegetable juices contained 72–93% of the protein content of the original tissues.

Experiments were carried out at the BIOENGINEERING DEPARTMENT OF THE CENTRAL FOOD RESEARCH INSTITUTE for the production of endo-polygalacturonase enzyme by submerged fermentation. The possibilities of application of the produced enzyme were investigated parallel to the fermentation work.

It was found that the enzyme is able to disintegrate plant and vegetable tissues resulting in vegetable suspensions utilizable either as vegetable juices and cocktails or vegetable pastes and baby food. It was necessary to determine how the enzyme treatment influences the nutritive value of the above products.

In this work, the protein content of vegetable juices and sieve fractions of the enzyme-treated vegetables (particles larger than 0.25 and 1.0 mm, resp.) was investigated. The vegetables tested were those, considered to be good raw materials for the production of vegetable juices and cocktails, namely carrots, red tomato-shaped paprika, green pepper, celery, red beet and squash.

1. Materials and methods

1.1. Varieties of vegetables tested

Carrots: *Chantenay, Vriflakk* and *Danro.*
Celery: *Frigga* and *Apia.*
Red beet: *Bíborgömb* (Purple orb) and *Egyiptomi lapos* (Egyptian flat).
Green pepper: *Cecei.*
Red tomato-shaped paprika: (Variety: *Early-ripe*).
Squash: *Nagydobosi.*

1.2. Enzyme preparation

Enzyme treatment was carried out with a liquid endo-polygalacturonase concentrate, produced by a strain of *Asp. awamori* (Pilot product of the CENTRAL FOOD RESEARCH INSTITUTE). Activity: 300 l h^{-1} ml^{-1}, determined viscosimetrically using Na polypectate (SERVA ENTWICKLUNGSLABOR, Heidelberg) as substrate (ZETELAKI & VAS, 1972). The protein content of the preparation was: 2.15%.

1.3. Preparation of vegetables for enzyme treatment and evaluation of the effects

Vegetables were grated or cut into pieces of 5×15 mm. Twenty-g quantities of the grated vegetables were placed into 250-ml Erlenmeyer flasks, 25 ml McIlvaine buffer (containing 0.05 g liquid enzyme preparation) were added to each and the reaction mixtures were incubated on a shaker (130 strokes min^{-1}) at 50 °C.

After the enzyme treatment, reaction mixtures were poured through a set of screens (mesh: first 1.0 and then 0.25 mm, resp.). Particles remaining on the screens were removed and dried at 105 °C to constant weight. Dry matter was determined together with the filtrate and the original tissue. The percentage of degradation into predominantly single cells and tissue particles (filtrate) and screen fractions larger than 0.25 and 1.0 mm, resp., was calculated from the dry weights.

Control samples were prepared the same way as the enzyme-treated ones, with the difference that no enzyme was added to the buffer.

The effect of enzyme treatment was investigated with enzyme concentrations of 0.1, 0.5 and 1.0% (related to the weight of the vegetable) and periods of incubation of 1.5 and 3 h. Thus, protein quantities of 0.0005, 0.0025 and 0.005 g were added to the reaction mixture with the above enzyme quantities.

Parameters of enzyme treatment were considered optimal when their use resulted in the highest protein portion of the original tissues, passing into the filtrate.

1.4. Determination of protein

Protein content was determined from the residue of the first screen (mesh 1.0 mm) and from the filtrate. The quantity of the residue remaining on the second screen (mesh 0.25 mm) was not enough for protein determination. The difference in protein quantity between the original tissue and that of the above two fractions was taken as protein content of the non-measurable fractions of the second screen.

Protein was determined by the modified (HERBERT *et al.*, 1971) biuret method.

1.4.1. Comparison of the biuret and the Kjeldahl methods. Protein contents of 70 samples of various vegetables were compared by using the biuret and the *Kjeldahl* methods (4 determinations from each). The results of the above investigation gave a coefficient of variation of $\pm 5.49\%$ for the *Kjeldahl* method and ± 6.76 for the biuret method.

The comparison of the results obtained by the above two methods is given in Figs. 1 and 2.

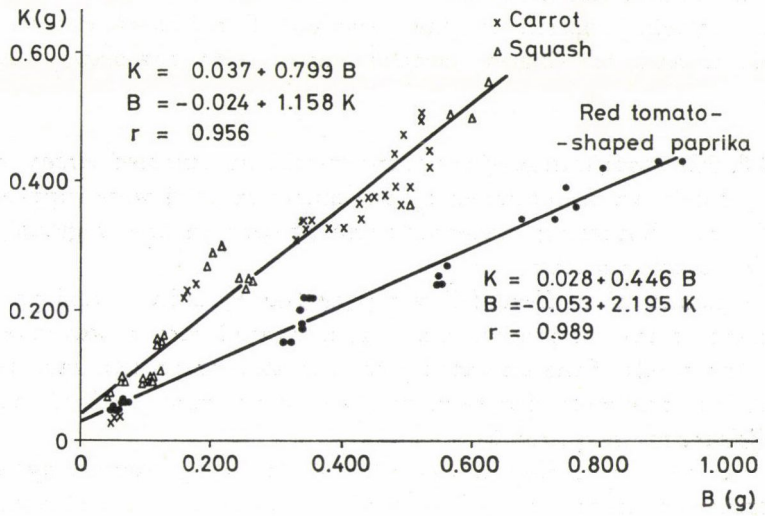

Fig. 1. Correlation between protein content data obtained by the *Kjeldahl* (K) and the biuret (B) method, in the case of whole carrots, squash and red tomato-shaped paprika (20-g samples)

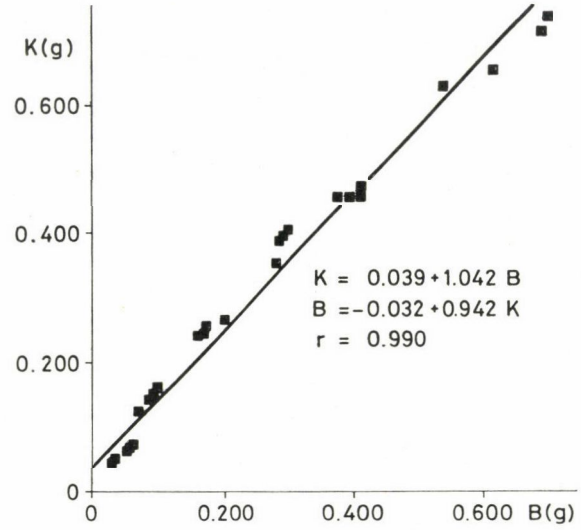

Fig. 2. Correlation between protein content data obtained by the *Kjeldahl* (K) and the biuret (B) method, in the case of whole red beet (20-g samples)

Protein content of the carrot, squash and red tomato-shaped paprika samples according to the biuret method proved to be higher than those obtained by the *Kjeldahl* method (Fig. 1).

When comparing the above two methods, the values of the slope calculated by correlation analysis, were 0.446 and 0.8022 in the case of red tomato-shaped paprika and carrots, resp.

The protein content in the samples of red beet determined by the *Kjeldahl* and the biuret methods proved approximately the same (Fig. 2).

1.4.2. The modification of the biuret method by activated carbon treatment. The high protein values obtained by the biuret method were supposed to be result of some disturbing materials being present in the vegetable tissues (probably colouring matter).

To eliminate this effect, following the NaOH addition and prior to the development of the Cu protein complex, activated carbon was given to the samples. As a result of the activated carbon treatment, protein values obtained by the photometric method were much lower and became similar to the results of the *Kjeldahl* method (Fig. 3).

As can be seen in the Figure, good results were obtained by activated carbon treatment in the case of red tomato-shaped paprika, squash, carrots. In the case of red beet, activated carbon treatment proved also useful, resulting in a decrease of the standard deviation of the methods.

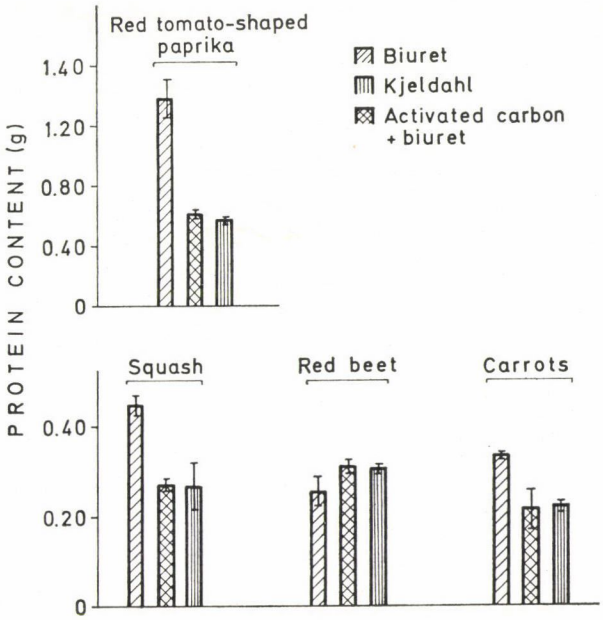

Fig. 3. Comparison of the *Kjeldahl* protein data with those of the biuret data with and without activated carbon treatment in various whole vegetables

1.4.3. Comparison of the various methods of protein determination by analysis of variance. Protein content of the various vegetables, determined by the *Kjeldahl*, biuret and modified (combined with activated carbon treatment) biuret methods were compared by analysis of variance (Table 1).

According to the F values, the differences among treatments proved to be very highly significant. The means of protein values obtained by the *Kjeldahl* method were compared also by the t-test to those of biuret and the modified biuret methods. It was found that the difference between the *Kjeldahl* and biuret methods was different at a very highly significant level in the case of every vegetable tested, while the results of the modified biuret method in no case differed from those of the *Kjeldahl* method.

2. Results

2.1. Protein solubilization in celery

The dry matter and protein contents of the various celery fractions are given as a function of enzyme concentration and time of incubation in Fig. 4. The increase in the time of incubation resulted in an increase in the dry matter and protein content of the filtrates. After a 1.5-h and 3-h incubation

Table 1

Comparison of the protein content of various vegetable juices as determined by the Kjeldahl *method on the one hand and by the biuret and the modified biuret methods on the other, using analysis of variance and the* t*-test*

Vegetable	DF	F	*t*-test for the differences between *Kjeldahl* data and those obtained with the methods indicated below	
			biuret	modified biuret
Squash	df_1: 2 df_2: 93 df_3: 95	500.90	*** 27.272	— 0.400
Carrots	df_1: 2 df_2: 45 df_3: 47	44.03	*** 11.936	— 0.157
Red beet	df_1: 2 df_2: 21 df_3: 23	9.60	*** 4.444	— 0.693
Red tomato-shaped paprika	df_1: 2 df_2: 9 df_3: 11	212.39	*** 10.880	— 1.060
Green pepper	df_1: 2 df_2: 9 df_3: 11	317.08	*** 20.570	— 0.715

*** Very highly significant.
— Not significant.

with 0.5% endo-PG, dry matter yields of 3.9 and 4.6 g were obtained in the vegetable juice of the *Apia* variety, while the solubilized protein increased also with the increase of the time of incubation from 1.6 g to 1.8 g, resp.

The increase in enzyme concentration increased the dry matter and protein contents of the filtrate and, at the same time, decreased the quantity of the 1st (>1 mm) and 2nd (>0.25) sieve fractions.

2.2. Protein solubilization in paprika and green pepper

The dry matter and protein contents of the various fractions of red tomato-shaped paprika and green pepper (variety: *Cecei*) are shown as a function of the time of incubation and of the enzyme concentration in Fig. 5.

In the case of green pepper, the increase in time of incubation from 1.5 to 3 h increased the dry matter recovery of the filtrate slightly but did not influence the protein release.

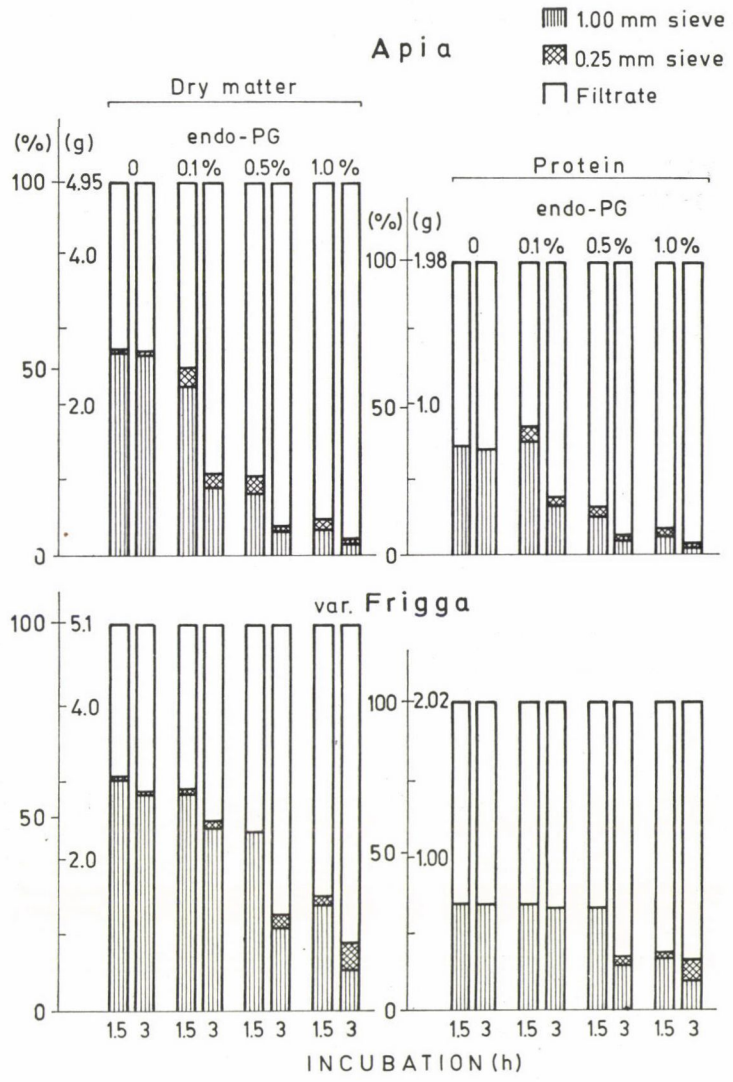

Fig. 4. Dry matter and protein distribution of celery varieties as a function of enzyme concentration and time of incubation, as measured in the various fractions of the ground vegetable (endo-PG conc.: 0.1, 0.5 and 1.0%; incubation: 1.5 and 3.0 h at 50 °C, pH: 3.0). Data relate to 20 g raw celery and are means of 2 treatments in 2 parallels each

When the concentration of endo-PG was increased from 0.1 to 0.5 and 1.0%, the dry matter yield in the filtrate increased by 0.17, 0.40 and 0.45 g, and the quantity of solubilized protein was increased by 0.08, 0.14 and 0.15 g after 3 h of incubation. The increase in enzyme concentration resulted in an increase in the quantity of the 2nd sieve fraction, too.

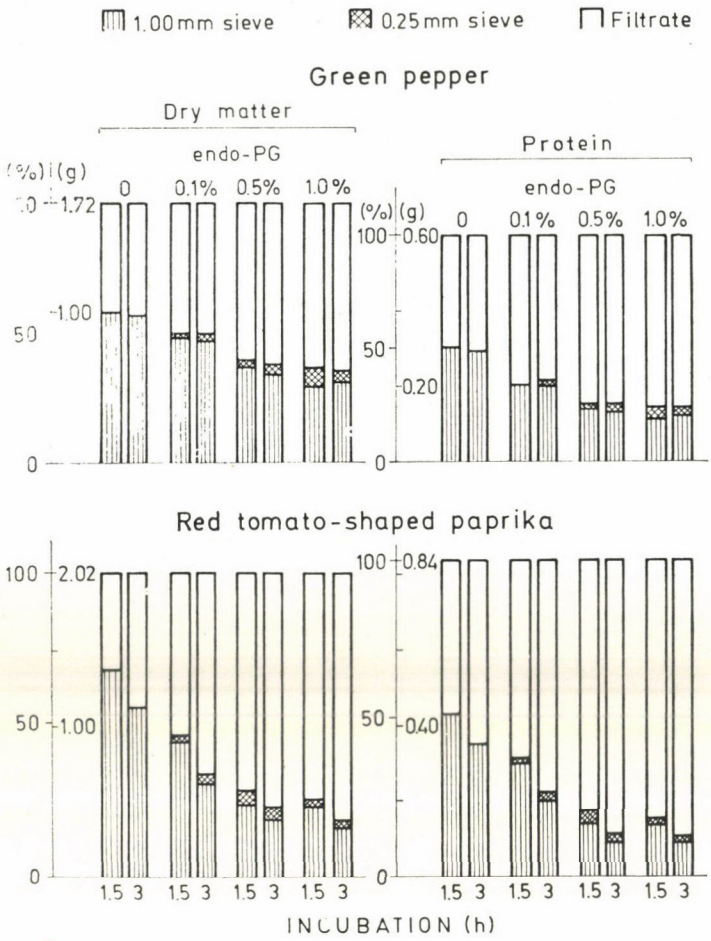

Fig. 5. Dry matter and protein distribution of paprika and green pepper as a function of enzyme concentration and time of incubation, as measured in the various fractions of the ground vegetable (endo-PG conc.: 0.1, 0.5 and 1.0%; incubation: 1.5 and 3.0 h at 50 °C, pH: 3.5 for red tomato-shaped paprika and pH: 4.5 for green pepper). Data relate to 20 g raw paprika or green pepper and are means of 2 treatments in 2 parallels each

In the case of red tomato-shaped paprika, the increase in the time of incubation slightly increased the dry matter and protein release to the filtrate, while the increase in enzyme concentration increased the dry matter yield and the solubilization of protein to a great extent.

0.53 g protein was attained with 0.1% endo-PG and 1.5 h of incubation while enzyme treatment with 1.0% of endo-PG for 3 h resulted in 0.74 g of protein.

2.3. Protein solubilization in carrots

The dry matter and protein contents of 3 varieties of carrots after endo-PG treatment are shown as a function of the time of incubation and enzyme concentration in Fig. 6.

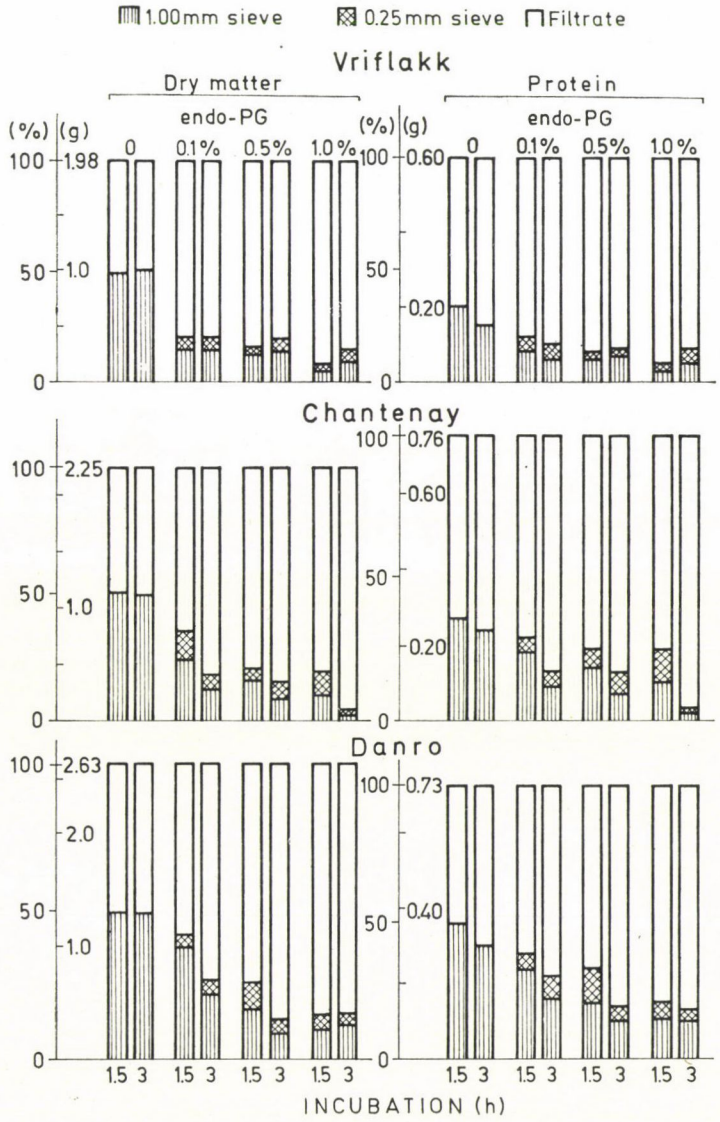

Fig. 6. Dry matter and protein distribution of carrots as a function of enzyme concentration and time of incubation, as measured in the various fractions of the ground vegetable (endo-PG conc.: 0.1, 0.5 and 1.0%; incubation: 1.5 and 3.0 h at 50 °C, pH: 3.5). Data relate to 20 g raw carrot and are means of 2 treatments in 2 parallels each

The increase of the time of incubation from 1.5 to 3 h increased the dry matter and protein contents in the filtrate of the *Chantenay* and *Danro* varieties, but in the case of the variety *Vriflakk*, 1.5 h of incubation was sufficient, resulting in a good enzymatic disintegration and, as a consequence, an increase in the protein content of the filtrate.

Of the enzyme concentrations used, 1.0% proved to be the best for each variety. An incubation period of 1.5 h gave better results with the *Vriflakk* variety, while for the *Chantenay* a 3-h incubation proved to be the most efficient.

2.4. Protein solubilization in red beet

Of the varieties of red beet, the *Biborgömb* (Purple orb) and the *Egyiptomi lapos* (Egyptian flat) were tested. Their dry matter and protein contents after enzyme treatment are given in Fig. 7.

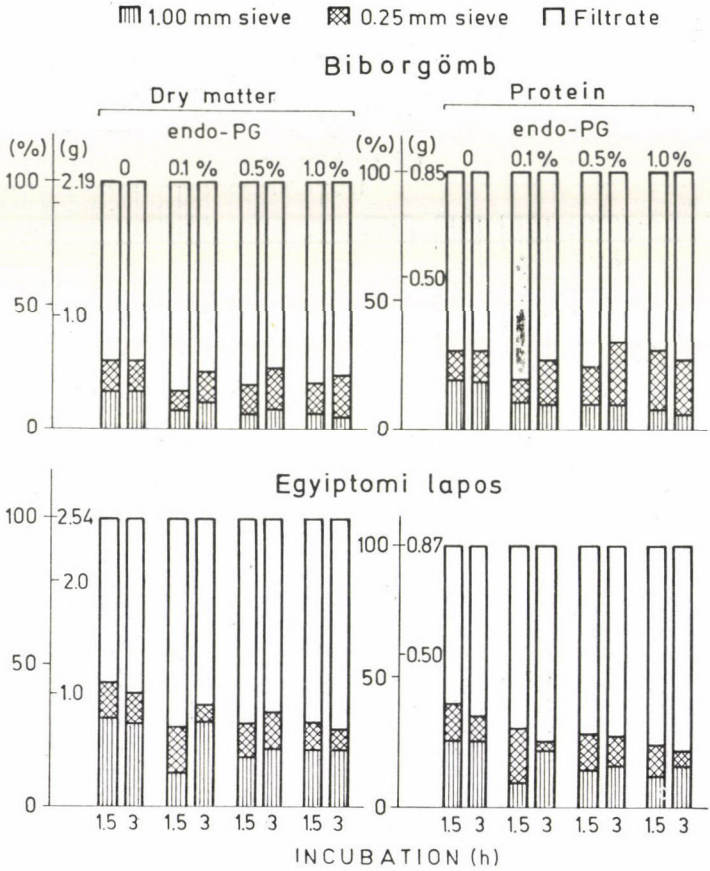

Fig. 7. Dry matter and protein distribution of red beet as a function of enzyme concentration and time of incubation, as measured in various fractions of the ground vegetable (endo-PG conc.: 0.1, 0.5 and 1.0%; incubation: 1.5 and 3.0 h at 50 °C, pH: 4.5). Data relate to 20 g raw red beet and are means of 2 treatments in 2 parallels each

The disintegrating effect of the enzyme for both varieties proved to be satisfactory after 1.5 h of incubation. The enzymatic disintegration was better and the dry matter and protein contents of the filtrate were higher in the case of *Bíborgömb* than those of the *Egyiptomi lapos* variety. It is apparent that the quantity of fine sieve fraction (0.25–1.00 mm) of the red beet samples was higher than in the case of the other vegetables tested. This might be the consequence of the precooking of red beet before enzyme treatment, to eliminate the unpleasant earthy taste.

In this case, the first sieve fraction increased with the increase of incubation time. This might be the consequence of aggregation of the disintegrated single cells and small particles. That is why the use of an incubation period longer than 1.5 h is not advisable in the endo-PG treatment of red beet.

2.5. Protein solubilization in squash

Nagydobosi variety of squash was used for the enzyme treatment where the increase in both the time of incubation and concentration of enzyme increased the dry matter and protein contents of the filtrate (Fig. 8).

As Fig. 8 clearly shows, the increase in the concentration of enzyme resulted in more efficient disintegration and, consequently, in more complete solubilization than the prolongation of the incubation period.

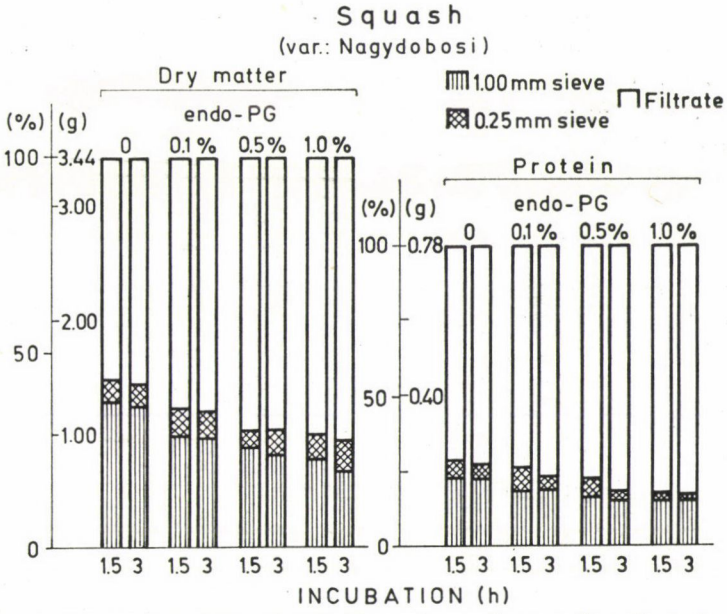

Fig. 8. Dry matter and protein distribution of squash as a function of enzyme concentration and time of incubation, as measured in various fractions of the ground vegetable (endo-PG conc.: 0.1, 0.5 and 1.0%; incubation: 1.5 and 3.0 h at 50 °C, pH: 4.5). Data relate to 20 g raw squash and are means of 2 treatments in 2 parallels each

3. Conclusions

Numerous experiments were carried out for the possible application of endo-PG enzyme (ZETELAKI-HORVÁTH & GÁTAI, 1977a, 1977b).

In the course of enzymatic disintegration of vegetables, the enzyme treatment improved the nutritional value of vegetable juices. Vitamin C, β-carotene and carbohydrate content of the vegetable juices increased as a consequence of enzyme treatment (ZETELAKI-HORVÁTH 1977; ZETELAKI-HORVÁTH *et al.*, 1978; GÁTAI & ZETELAKI-HORVÁTH, 1978). To investigate the effect of enzyme treatment on vitamin, protein and carbohydrate content of the juices a huge amount of analyses had to be done simultaneously.

The aim of this work was to evaluate the protein release of vegetable tissues after enzyme treatment. A quick method (modified biuret, HERBERT *et al.*, 1971) was chosen to determine the protein in enzyme-treated vegetables (sieve fractions, filtrates, and the original tissues) for the substitution of the labour- and time-consuming *Kjeldahl* method. The two method were compared.

In the course of our work, the yellow colour of several vegetables (carrots, squash and paprika) was also found to interfere with the method. Results of protein determination by the biuret method were in agreement with the *Kjeldahl* results only when biuret was complemented with an active carbon treatment, following the addition of NaOH. The red colour of red beet caused no interference, though active carbon treatment decreased the standard deviation of the method.

These are in agreement with the results of HERBERT and co-workers (1971), who stated that glucose and other reducing sugars may interfere, owing to their caramelization and the formation of a strong yellow colour. The dry matter and protein content of the filtrates obtained after endo-PG treatment of vegetables showed a remarkable increase compared with those of the control samples (the yields of the control samples were taken as 100%, Fig. 9).

The dry matter content of the juices after enzyme treatment of vegetables were 100% higher in the case of carrot and celery (varieties: *Chantenay* and *Apia*) and 80% higher in the case of red tomato-shaped paprika and celery, variety: *Frigga*.

The protein content of the juices of red tomato-shaped paprika, and of green pepper (variety: *Cecei*) were about 60 and 50% higher, resp., after enzyme treatment (at optimal conditions), while in the case of carrots (variety: *Chantenay*) and celery (variety: *Apia*) about 40% higher.

All these results were obtained with 1% enzyme preparation of an endo-PG activity of 300 $l\ h^{-1}\ ml^{-1}$, while in the case of red beet, a protein release of 30–35% higher than that of the control was obtained with a concentration of 0.1% of the same enzyme preparation.

The protein content of the juices of all the enzyme-treated vegetables was highly significantly (0.1% endo-PG) and very highly significantly (0.5 and 1.0% endo-PG) higher than those of the control when compared by mathematical statistical evaluation.

The increase in dissolved protein content of vegetable juices must partly be the result of the disintegrating effect of the enzyme treatment. In this case,

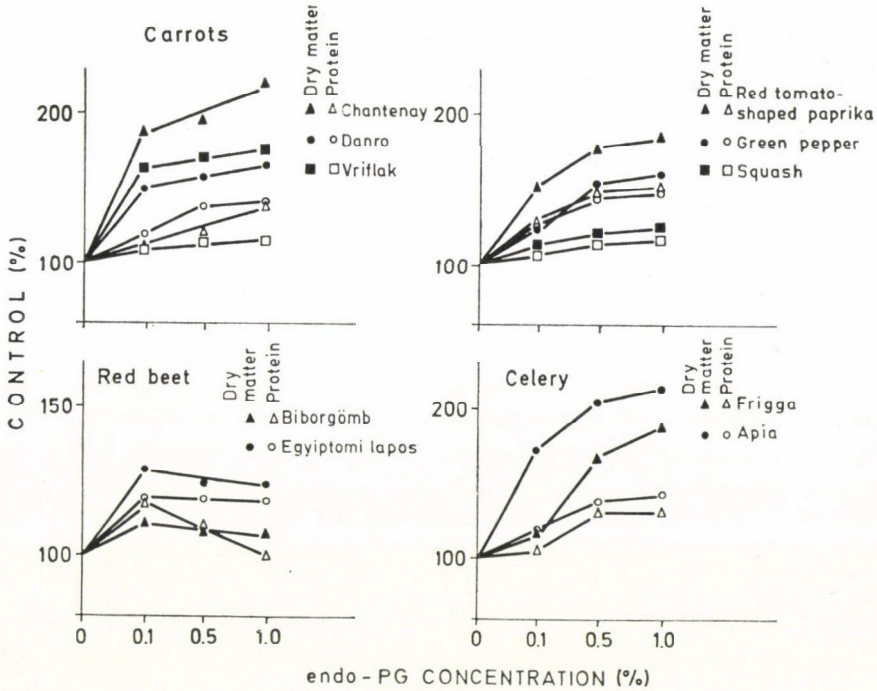

Fig. 9. Dry matter and protein release to the filtrates of various vegetables as a function of enzyme treatment (endo-PG conc.: 0.1, 0.5 and 1.0%; incubation at 50 °C, at optimum pH for 3.0 h for red tomato-shaped paprika, green pepper, squash and carrot (var.: *Chantenay*) and a 1.5 h period for celery, red beet and carrots (var.: *Danro, Vriflakk*)

almost the whole tissue gets into the juice, while in the control samples a great amount remains on the screens.

Furthermore, a better release of cell wall proteins may also occur as a consequence of endo-PG treatment. STRAND and co-workers (1976) stated that half of the total measured cell-wall proteins of several vegetables was released by pectic enzymes. These results prove that galacturonides are important links between wall proteins and the cell-wall. Thus, disintegration of pectic materials results not only in solubilization of cell-wall proteins of broken cells, but may also increase the permeability of cell-walls, and, as a consequence, the diffusion of proteins from the intact cells.

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A NEW EXPERIMENTAL METHOD FOR THE DETERMINATION OF THE HEAT DESTRUCTION PARAMETERS OF MICROORGANISMS

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The essence of the new experimental method lies in the fact that both the heat destruction curve and the *Arrhenius* plot demonstrating the temperature dependence of the death rate coefficient may be constructed as the result of a single series of measurements carried out in a single system. The method is equally suitable for heat destruction tests above and below 373 K (100 °C).

By continually increasing and measuring the temperature of the suspension of microbes to be tested samples are taken at predetermined intervals and these are plated to determine the viable cell count belonging to the given points of time. The logarithm of the viable cell count plotted against time gives a curve of increasing slope. This increase in the slope of the survival curve is due to the value of the death rate coefficient increasing with increasing temperature.

The decimal reduction time values or the death rate coefficients belonging to a given point of time may be calculated from the differential quotient with respect to time of the survival curve. The logarithm of decimal reduction times plotted against the temperature values belonging to the appropriate points of time gives the heat destruction curve. The *Arrhenius* plot may be constructed by plotting the logarithm of the death rate coefficients as a function of the reciprocal of the absolute temperature.

The advantage of the method over traditional heat destruction tests lies in the fact that during the measurements neither the age of the culture nor the composition of the heat treatment medium or of the culturing medium change and thus all kinds of interfering factors may be eliminated. A further advantage is that the method adequately simulates practical sterilization procedures involving varying temperatures and thus the results obtained serve as safe basis in the calculation of the sterilization equivalent.

The tests were carried out so far with cultures of varied age of *Saccharomyces cerevisiae*, *Escherichia coli* and *Bacillus stearothermophilus*. The death rate coefficients, decimal reduction times and the z values as determined by this and the traditional method and compared to data of the literature proved the applicability of the new method.

The customary heat destruction tests applied in practice have in common that the death rate coefficient or the decimal reduction time (D value) is determined at different temperatures in several experiments and the heat destruction curve is constructed from the data thus obtained.

In determining the D value it is generally assumed that the decrease in viable cell count with time is purely exponential and the evaluation methods are based on this assumption. However, according to experience, the death rate may be described by the first order kinetics of chemical reactions only at a certain level of cell density or in a certain time interval. Figure 1

illustrates the characteristic types of the survival curves deviating from first order kinetics.

Curves of these types are frequently found in the literature on the death of bacterial spores as reviewed by RUSSEL (1971).

SHULL and ERNST (1962) and COOK and BROWN (1965), while investigating heat destruction of *B. stearothermophilus*, described in detail the shape of the survival curve resembling curve A in Fig. 1; the exponential shape may be observed after an initial heat activation period.

A survival curve with a resistant tail, similar to curve B in Fig. 1, was observed in the case of *B. cereus* (VAS & PROSZT, 1957) and *Cl. botulinum* (ROBERTS & INGRAM, 1965). However, a survival curve similar to curve B is observable not only with bacterial spores. An identical shape was found by VERRIPS and KWAST (1977) with *Citrobacter freundii* and by MOATS and coworkers (1971) with *Escherichia coli*.

A survival curve of type C in Fig. 1 was found with *Escherichia coli* (RUSSEL & HARRIES, 1968) and some yeast strains (CORRY, 1976) when studying their thermal death.

If the method used for determining the D value is based on the enumeration of initial and final viable cell counts, the value thus calculated and the death rate coefficient may be extremely misleading due to transient phases of the survival curve. One of the methods is the use of the formula of STUMBO (1948):

$$D = \frac{U}{\log N_o - \lg N_u}$$

where D is the decimal reduction time
U is the period of heat treatment
N_o the initial viable cell count
N_u the viable cell count after time U.

Taking into account that N_u is frequently calculated by the *Halvorson* formula (HALVORSON & ZIEGLER, 1933) the reliability of the method is questionable. In the case of the *Halvorson* formula:

$$N_u = \frac{2.303}{a} \cdot \lg \frac{r}{q}$$

where

N_u viable cell count per ml
a volume of the heat-treated suspension (ml)
r number of heat treated samples
q number of samples showing lack of growth.

The methods taking into account only the exponential death phase as determined from the viable cell count at different points of time facilitate a

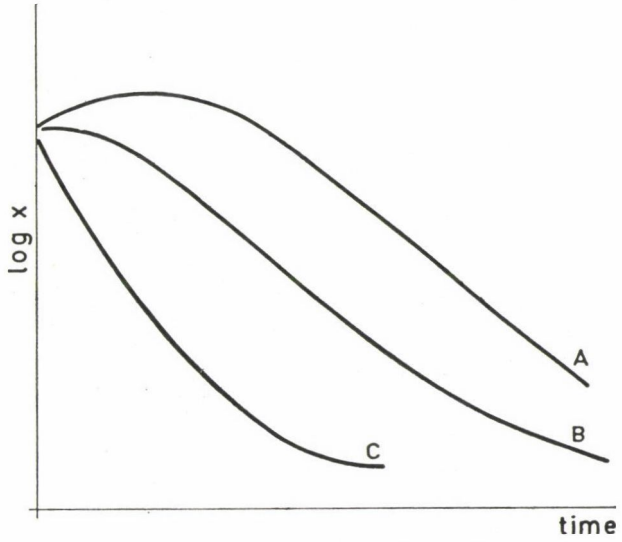

Fig. 1. Characteristic survival curves deviating from first order kinetics, indicating viable cell count (x) as a function of treatment time

more exact determination of the death rate coefficient or the D value. Since in the case of first order kinetics:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = -\mathrm{k} \cdot x,$$

thus

$$k = -2.303 \frac{\mathrm{d} \lg x}{\mathrm{d}t},$$

and

$$D = \frac{2.303}{k},$$

where

x viable cell concentration
t time
k the death rate coefficient
D the decimal reduction time.

The method is illustrated in Fig. 2.

The values of k and D may be calculated from the slope of the linear phase of the survival curve.

It is relatively easy to measure the survival curve with heat treatments below 373 K (100 °C) in an aqueous medium. For temperatures above this, a very simple and reliable method was developed by KOOIMAN and GEERS

(1974): the screw-capped tube technique. The main feature of the method is that the suspension of microbes to be investigated is injected into plastic-capped tubes containing the heat treatment medium and placed in a thermostated glycerol bath. The proportion of the injected quantity and the thermo-

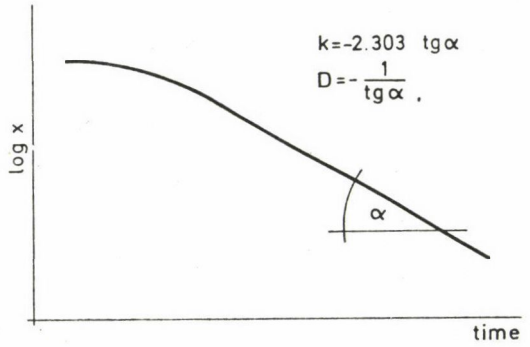

Fig. 2. Determination of decimal reduction time (D) and death rate coefficient (k) from the linear section of a survival curve (x = viable cell count)

stated heat treatment medium is 1 : 100. As the injected suspension is very small, it reaches the desired temperature in a few seconds only.

However, thermal death tests carried out at different temperatures may contain disturbing factors (HANSEN & RIEMANN, 1963). Apart from the age of the culture, such a factor is the heat treatment medium (CORRY, 1974; CORRY, 1976; VERRIPS & KWAST, 1977) and the differences of composition in the water activity and pH of the medium (COOK & BROWN, 1965; RUSSEL & HARRIES, 1968).

Thermal death determinations at different temperatures have, besides numerous sources of error, high material and instrument requirements.

The aim of this study was to eliminate the above-mentioned drawbacks by a new approach.

1. Materials and methods

1.1. Microbial strains

1.1.1. Saccharomyces cerevisiae. Strain KE 150 obtained from the stock culture collection of the Microbiological Gene Bank, Budapest.

Maintaining medium: 4% glucose, 1% peptone, 1% yeast extract, 2% agar; pH = 5.8.

Storage temperature: 288 K (15 °C).

Cultivation temperature: 303 K (30 °C).

1.1.2. Escherichia coli. Strain B 200 from the stock collection of the Microbiological Gene Bank, Budapest.

Maintaining medium: 0.1% glucose, 0.5% peptone, 0.25% yeast extract, 2% agar; pH = 7.0.

Storage temperature: 288 K (15 °C).

Cultivation temperature: 310 K (37 °C).

1.1.3. Bacillus stearothermophilus. The strain was maintained in the Microbiological Department Group of the University of Horticulture.

For maintainance and storage see 1.1.2.

Cultivation temperature: 328 K (55 °C).

1.2. Heat treatment medium

As heat treatment medium a sterile 0.5% glucose solution of pH 4.7 was used throughout, except in the 1st *B. stearothermophilus* experiment where glycerol was used.

1.3. Preparation of the suspension

Four slant cultures were washed down with 0.5% glucose solution. The washings were made up with 0.5% glucose solution to 400 ml, in the case of *E. coli* and *S. cerevisiae*, and to 30 ml, with *B. stearothermophilus*. In experiments with *E. coli* and *S. cerevisiae* the whole of the 400 ml was heat-treated. Thermal destruction of *B. stearothermophilus* was tested in two different ways. In the first case, the total amount of suspension was mixed with 200 ml of glycerol; in other experiments 0.1- or 0.2-ml portions were transferred to small glass tubes and these were heat treated in glycerol medium. Spore formation of *B. stearothermophilus* was checked under a phase contrast microscope. The cultures used in the experiments were sporulated nearly 100%.

1.4. Glass tubes used for heat treatment

To test *B. stearothermophilus* at temperatures above 373 K (100 °C) in aqueous medium, amounts of 0.1 or 0.2 ml suspension were injected into glass tubes and these were sealed straight away. The tubes were of 5 mm in diameter, and 40 mm in length, their walls were 0.2 mm thick. The small tubes were fixed in the glycerol bath with copper wire so as to be covered by the glycerol.

1.5. Determination of the viable cell count

The heat treated samples were diluted and each dilution was plated. Sterile 9 ml peptone water was used for dilution. The nutrient and the cultiva-

tion temperature was the same as described in Section 1.1. For the propagation of *S. cerevisiae* the pH of the nutrient was set at 4.0 by applying sterile tartaric acid. The colonies were counted after 3 days of incubation. The number of colonies in different dilutions was used for the calculation of cell count per ml taking their logarithmic means.

1.6. Methods

1.6.1. Traditional method. The thermal death of *E. coli* and *S. cerevisiae* was determined by the traditional method. The aim was to compare the results obtained by the traditional method with those obtained by the new experimental method.

Four slant agar cultures were washed down with 10 ml glucose solution and this dense suspension was added to 390 ml 0.5% glucose solution of appropriate temperature. The temperature was ensured by circulating hot water through a heating spiral and by constant agitation. The temperature of the water used for heating was kept at a constant level with a *Medingen* U 10 ultrathermostat. A *Radelkis* OP 912/3 type magnetic stirrer was used for agitation. At appropriate intervals, samples were taken from the beaker containing the suspension to count viable cells. The D value was calculated from the regression equation of the linear section of the survival curve.

1.6.2. The new method. This method permits the construction of the thermal death curve on the basis of a single series of measurements in the same system.

The temperature of the suspension to be studied is continuously altered and the parameters are calculated from the viable cell counts determined point by point. Since during the study neither the age of the culture, nor the experimental medium, nor the composition of the growth medium change, the results can be accounted for by the changes caused by varying the temperature.

Since the death rate coefficient increases exponentially with the increase in temperature it is expedient to set up the rate of temperature increase exponentially decreasing with time. Thus it is possible to achieve that the death rate coefficient increases nearly linearly with time. Thereby the measurement takes more time and becomes more exact.

A temperature increase exponentially decreasing with time may be produced if in a thermologically first order system – *i.e.* in a well insulated and perfectly agitated container – the temperature of the heating medium is suddenly increased. In this case the temperature of the container, in accordance with the transient function of first order elements, changes as required above:

$$T_t = T_o + (T_f - T_o) \cdot (1 - e^{-t/C}),$$

where

T_t is the temperature of the container at time t

T_o is the temperature of the container at o point of time (at the starting time of temperature change)

T_f is the temperature of the heating medium

C is the thermological time constant of the container

$$C = \frac{c_p \cdot V \cdot d}{K \cdot F},$$

where

c_p is the specific heat of the liquid in the container at constant pressure

V is the volume of the liquid

d is the density of the liquid

K is the heat transfer coefficient

F is the surface of heater.

No matter how the time constant is determined, experimentally or by calculation, it allows the design of the thermal profile. By the appropriate selection of the thermal profile, every sterilization process of varying temperature may be modelled. The knowledge of the time constant permits the numerical optimisation of the method, that is, given the inaccuracies of cell counting and time measurement, the $(T_f - T_o)$ value may be calculated at which the error committed in the determination of z is minimal. (The z value is the negative reciprocal of the slope of the thermal destruction curve; it measures the change in temperature required to bring about a tenfold change in the rate of thermal destruction.)

A beaker of 400 ml useful volume was used in the experiment. Its contents were agitated with a *Radelkis* OP 912/3 type magnetic stirrer consisting of a Teflon-coated magnetic core. The temperature of the heat transmitting medium was controlled with a heating spiral, the temperature of which was maintained at the desired level by a thermostat of the type U–10. To measure the temperature, a mercury-filled thermometer subdivided into 0.2 °C was used.

The time constant of the instrument, using 400 ml of an 0.5% glucose solution as a medium was appr. 300 s, the time constant of the thermometer 4–6 s, both experimentally determined. The great difference between the two time constants renders the thermometer suitable for measuring the temperature of the medium.

At temperatures below 373 K (100 °C) the 400 ml glucose solution containing the microbes was poured into the beaker and was then thoroughly mixed for 10 min at room temperature. The water used for heating to 343 K (70 °C), was then made to circulate.

As soon as the desired temperature had been reached the timer was started and the temperature was read and a sample of 1.0 ml was taken every

0.5 min. The sample was immediately cooled down by pipetting it into the first member of the dilution series.

Heat treatment above 373 K (100 °C) of the *B. stearothermophilus* was carried out in the same equipment but in this case both the heat transfer and the heating media consisted of glycerol. In the first experiment the *B. stearothermophilus* culture was suspended directly in glycerol and it was treated exactly like *E. coli* and *S. cerevisiae* at temperatures below 373 K (100 °C). However, in subsequent experiments the samples suspended in 0.5% glucose were heat treated in sealed glass tubes. Under constant agitation, the heat transfer medium of 423 K (150 °C) was started to circulate and the temperature was read every 0.5 min. Simultaneously, two suspension-containing glass tubes were removed and immediately cooled in cold water. After sterilizing the outer surface of the tubes with alcohol and drying them they were broken and their contents transferred to the first member of the dilution series.

Since at low cell concentrations the spores or cells introduced on the surface of the tubes or originating, *a.o.*, from tubes exploded in the heat transfer medium, may interfere with counting, tubes containing only 0.5% glucose solution were placed in the glycerol bath. In determining cell counts only the values greater than those obtained with the control tubes were taken into account. The lower limit of cell density which can be determined by this method was *cca.* 10^2 ml^{-1}, since 0.1 ml was added to the first member of the dilution series of 9 ml.

1.7. Evaluation of results

The results obtained by the new method were treated as follows:

The temperature and the logarithm of the viable cell count were plotted against time then the best fitting curves were drawn. The values read from the curves served for calculation. The slope of the survival curve was calculated point by point and from this the death rate coefficient and the D value were obtained. The temperature belonging to a given time was interpolated and the logarithm of the D value was plotted as a function of this. Thus the points of the thermal destruction curve were obtained and the z value was calculated by regression analysis. By plotting the logarithms of the death rate coefficients as functions of the reciprocal of the absolute temperature, the points of the Arrhenius plot were obtained. The regression equation of this correlation was used to calculate the apparent activation energy (E) of the process of destruction.

The evaluation procedure is illustrated in detail by one of the experiments with *E. coli* as follows.

The changes of viable cell count and temperature in the course of time are illustrated in Fig. 3.

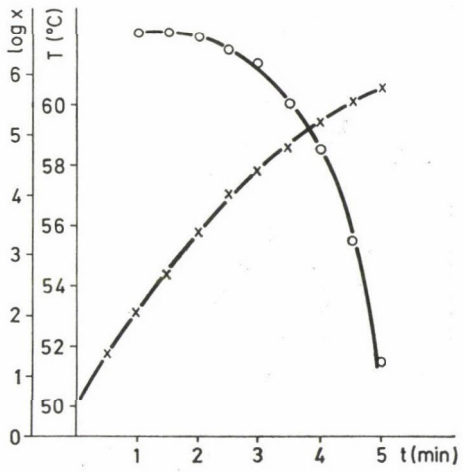

Fig. 3. Changes in temperature (T) and viable cell count (x) of *E. coli*, Series 1 in thermal destruction experiments. Heat treatment medium: 0.5% glucose solution. Age of culture: 1 week

— ○ — ○ — Viable cell count
— × — × — Temperature

The treatment of data, and steps of calculation are summarized in Table 1.

Plotting the lg D values as a function of the interpolated temperature data (T_i) the points of the thermal destruction curve are obtained, as shown in Fig. 4.

Table 1

Evaluation of experimental thermal destruction results in Series 1 with E. coli

t (min)	T_m* (°C)	lg x	T_i** (°C)	T_i** (K)	$\frac{-\mathrm{d}\lg x}{\mathrm{d}t} = k'$ (min^{-1})	$D = \frac{1}{k'}$ (min)	lg D	$k = 2.303\,k'$ (min^{-1})	lg k	$\frac{1}{T} \cdot 10^4$ (K^{-1})
1.5	54.4	6.68	55.1	328.1	0.14	7.14	0.854	0.322	—0.492	30.48
2.0	55.8	6.61	56.4	329.4	0.36	2.78	0.444	0.829	—0.081	30.36
2.5	57.0	6.43	57.4	330.4	0.64	1.56	0.193	1.474	0.168	30.27
3.0	57.8	6.11	58.2	331.2	1.06	0.943	—0.025	2.44	0.387	30.19
3.5	58.6	5.58	59.0	332.0	1.64	0.610	—0.215	3.78	0.577	30.12
4.0	59.4	4.76	59.8	332.8	2.92	0.342	—0.466	6.72	0.827	30.05
4.5	60.2	3.30	60.4	333.4	4.60	0.217	—0.664	10.59	1.025	30.00

* T_m = Measured values
** T_i = Interpolated values

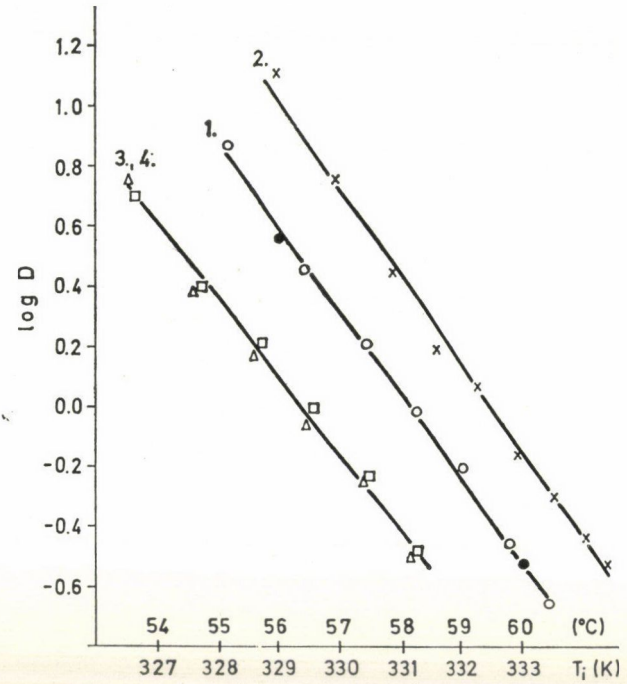

Fig. 4. Thermal death curves, belonging to different experimental series with *E. coli*, showing decimal reduction time (D) as a function of treatment temperature (T_i = interpolated temperature)

— ○ — ○ — *E. coli*, Series 1
— × — × — *E. coli*, Series 2
— △ — △ — *E. coli*, Series 3
— □ — □ — *E. coli*, Series 4
— ● — ● — *E. coli*, Series 1, data obtained by the traditional method

Of the corresponding lg D and T data pairs the equation of the straight line is calculated by linear regression. The negative reciprocal of the slope gives the z value (1st row in Table 3).

Plotting the logarithm of the death rate coefficient (k) against the reciprocal of temperature, the Arrhenius curve is obtained (1st curve in Fig. 5).

The apparent activation energy of the destruction process is calculated from the slope of the straight line:

$$\operatorname{tg} \alpha = \frac{-E}{2.303 \cdot R}$$

where E is the apparent activation energy
R is the universal gas constant.

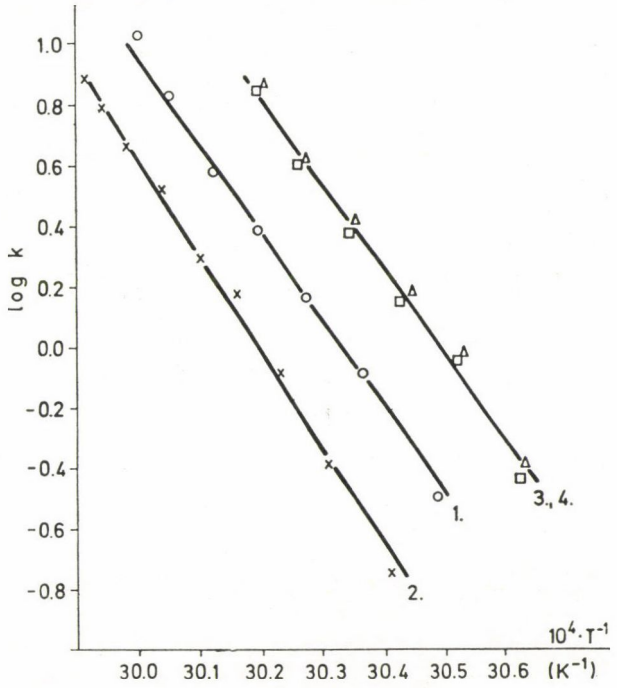

Fig. 5. Arrhenius plots of the death rate coefficients (k) determined as a function of interpolated temperature (T_i) in experiments with *E. coli*
— ○ — ○ — *E. coli*, Series 1
— × — × — *E. coli*, Series 2
— △ — △ — *E. coli*, Series 3
— □ — □ — *E. coli*, Series 4

2. Results

2.1. Thermal death of E. coli

The experiments were carried out with cultures of different ages and stored at different temperatures.

Series 1: *E. coli* cultivated at 310 K (37 °C) and stored for one week at room temperature.

Series 2: *E. coli* cultivated at 310 K (37 °C) for 24 h.

Series 3 and 4: *E. coli* cultivated at room temperature for 24 h.

The results are given in Tables 1 and 2.

The thermal destruction curves of cultures kept under different conditions are shown in Fig. 4. The death rate coefficients are illustrated as a function of temperature in Fig. 5. Since the cultures in Series 3 and 4 were kept

Table 2

Evaluation of experimental thermal destruction results in Series 2, 3 and 4 with E. coli

2			3			4		
T_i (K)	D (min)	k (min^{-1})	T_i (K)	D (min)	k (min^{-1})	T_i (K)	D (min)	k (min^{-1})
328.9	12.5	0.184	326.5	5.55	0.414	326.6	6.25	0.368
329.9	5.56	0.414	327.55	2.38	0.966	327.7	2.50	0.920
330.8	2.78	0.827	328.55	1.47	1.56	328.7	1.61	1.43
331.55	1.52	1.51	329.45	0.862	2.67	329.6	0.980	2.35
332.25	1.16	1.98	330.35	0.556	4.14	330.45	0.568	4.05
332.9	0.694	3.31	331.10	0.312	7.37	331.25	0.323	7.12
333.5	0.505	4.55						
334.0	0.370	6.22						
334.35	0.298	7.72						

under identical conditions their data were evaluated and illustrated after pooling.

The equation of thermal destruction curves, the z values, the apparent activation energies and their confidence intervals, resp. are summarized in Table 3.

The experiments with *E. coli* were carried out also by the traditional method. The D values obtained were as follows:

at **329 K** (56 °C) $D = 3.54$ min
at **333 K** (60 °C) $D = 0.30$ min.

Table 3

Equations of thermal destruction curves, z values and apparent activation energies in experiments with E. coli

Series	No. of data pairs	Thermal destruction curve	r*	z (K)	$z_{95\%}$** (K)	E (J mol^{-1})
E. coli 1.	7	$\lg D = 92.37 - 0.2791 \cdot T$ (328 K — 334 K)	—0.999	3.58	3.39—3.80	$(5.46 \pm 1.64) \cdot 10^5$
E. coli 2.	9	$\lg D = 97.07 - 0.2922 \cdot T$ (328 K — 334 K)	—0.997	3.42	3.18—3.70	$(6.11 \pm 0.45) \cdot 10^5$
E. coli 3. 4.	12	$\lg D = 86.9 - 0.2614 \cdot T$ (326 K — 331 K)	—0.993	3.83	3.57—4.13	$(5.22 \pm 0.98) \cdot 10^5$

* r = Correlation coefficient

** $z_{95\%}$ = The 95% confidence interval of the z value. (Calculated from the confidence interval of the slope of the thermal destruction curve)

The results obtained by the traditional method are illustrated by Curve 1 in Fig. 4. It can be seen that the results closely fit those obtained by the new method.

2.2. *Thermal death of* S. cerevisiae

The experiments were carried out with cultures stored at the same temperature for different periods of time.

Series 1 and 2: *S. cerevisiae* stored for 24 h at 303 K (30 °C).

Series 3 and 4: *S. cerevisiae* stored for 168 h at 303 K (30 °C).

Changes in temperature and cell density are illustrated in Fig. 6 (Series 1) and in Fig. 7 (Series 3). Results are given in Table 4.

Table 4

Results of thermal destruction experiments with S. cerevisiae

1			2			3			4		
T_i (K)	D (min)	k (min^{-1})	T_i (K)	D (min)	k (min^{-1})	T_i (K)	D (min)	k (min^{-1})	T_i (K)	D (min)	k (min^{-1})
324.3	6.25	0.368	325.2	4.55	0.506	324.1	8.34	0.276	323.45	10.0	0.230
325.4	3.33	0.692	326.2	2.38	0.968	325.35	3.33	0.692	324.84	5.00	0.461
326.35	1.85	1.24	327.1	1.25	1.84	326.55	1.51	1.53	326.15	2.38	0.968
327.25	1.09	2.11	327.85	0.714	3.23	327.6	1.22	1.89	327.3	1.35	1.71
328	0.694	3.32	328.55	0.385	5.98	328.55	0.667	3.45	328.25	0.834	2.76
328.65	0.417	5.52				329.4	0.500	4.61	329.15	0.612	3.76
						330.15	0.417	5.52	330.0	0.430	5.36

The corresponding lg D–T data pairs are shown in Fig. 8, while the death rate coefficients *vs.* T^{-1} in Fig. 9.

With 24-h cultures, the thermal destruction curve (Fig. 8) may be described by a straight line. The thermal death curves belonging to 168-h cultures fall into two sections: below 327 K (54 °C) the thermal death data correspond exactly to those of the 24-h cultures. Above 327 K (54 °C) the thermal death curve as obtained in Series 3 and 4 of *S. cerevisiae* can be described by a line of different slope. The latter section is illustrated by Line a in Fig. 8. Obviously, the same may be seen in Fig. 9 in relation to the Arrhenius plot.

The equations of the thermal destruction curves, the values z and E, and their confidence intervals are given in Table 5.

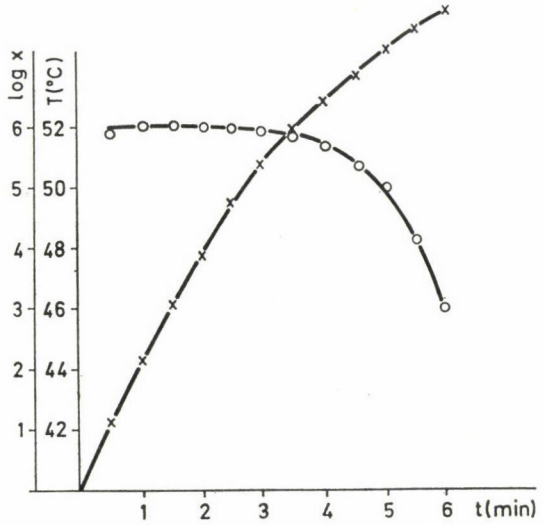

Fig. 6. Changes in viable cell count (x) and temperature (T) as a function of treatment time (t) in experiments with *S. cerevisiae*, Series 1. Heat treatment medium: 0.5% glucose solution. Age of culture: 24 h

— ○ — ○ — Viable cell count
— × -- × — Temperature

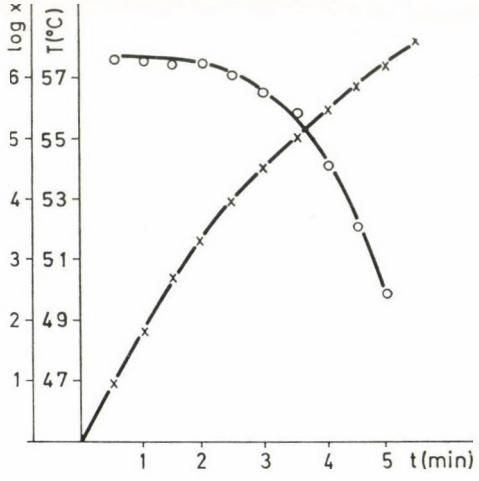

Fig. 7. Changes in viable cell count (x) and in temperature (T) as a function of treatment time (t) in experiments with *S. cerevisiae*, Series 3. Heat treatment medium: 0.5% glucose solution. Age of culture: 168 h

— ○ — ○ — Viable cell count
— × — × — Temperature

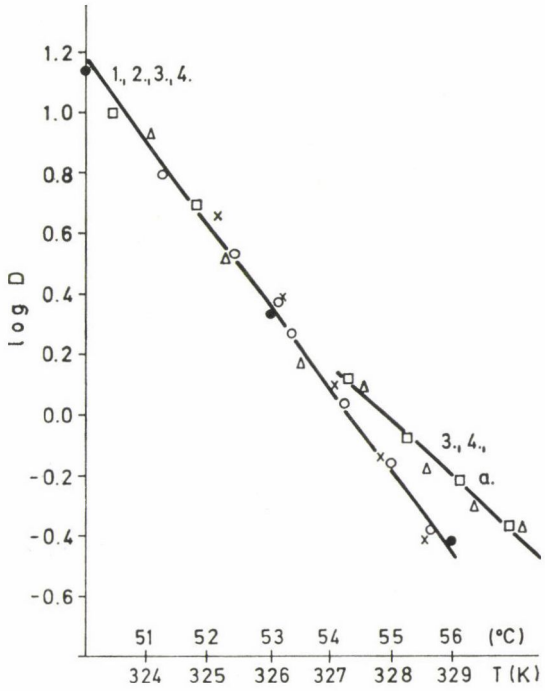

Fig. 8. Thermal death curves obtained in *S. cerevisiae* experiments showing decimal reduction time (D) as a function of interpolated temperature (T_i) (For details, see the text)

— ○ — ○ — *S. cerevisiae*, Series 1
— × — × — *S. cerevisiae*, Series 2
— △ — △ — *S. cerevisiae*, Series 3
— □ — □ — *S. cerevisiae*, Series 4
— ● — ● — Values obtained by the traditional method

Table 5

Equations of thermal destruction curves, z values and apparent activation energies in S. cerevisiae *experiments*

Series	Number of data pairs	Thermal death curve	r	z (K)	$z_{95\%}$ (K)	E (J mol^{-1})
Sacch. cer. 1, 2, 3, 4*	14	$\lg D = 90.38 - 0.2761 \cdot T$ (323 K — 329 K)	−0.995	3.62	3.45—3.82	$(5.62 \pm 0.36) \cdot 10^5$
Sacch. cer. 3, 4**	8	$\lg D = 59.74 - 0.1822 \cdot T$ (327 K — 330 K)	−0.987	5.49	4.73—6.53	$(3.75 \pm 1.60) \cdot 10^5$

* Calculated from all data belonging to Series 1 and 2 and those below 327 K (54 °C) of Series 3 and 4

** Calculated from data above 327 K (54 °C) of Series 3 and 4

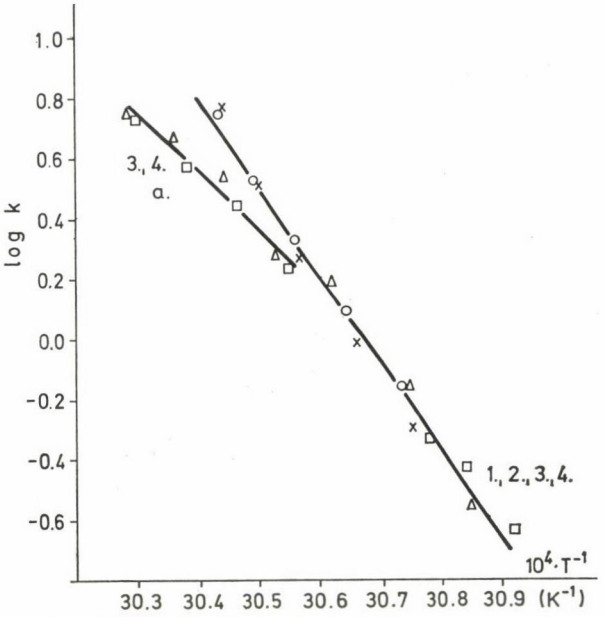

Fig. 9. *Arrhenius* plots of death rate coefficients (k) obtained as a function of interpolated temperature (T_i) in experiments with *S. cerevisiae*

— ○ — ○ — *S. cerevisiae*, Series 1
— × — × — *S. cerevisiae*, Series 2
— △ — △ — *S. cerevisiae*, Series 3
— □ — □ — *S. cerevisiae*, Series 4

The D values belonging to the 24-h cultures were determined at 3 temperatures by the traditional method, too. The results can be summarized as follows:

at 323 K (50 °C)	$D = 13.4$ min,
at 326 K (53 °C)	$D = 2.17$ min,
at 329 K (56 °C)	$D = 0.38$ min.

The data are illustrated in Fig. 8. As may be seen, the results fit those obtained by the new method very well.

2.3. *Thermal destruction of* B. stearothermophilus

2.3.1. Study of thermal destruction in glycerol medium. In the temperature range up to 420 K (147 °C) no substantial death could be observed. Figure 10 shows the temperatures and the count of surviving cells.

2.3.2. Thermal destruction in 0.5% glucose solution. Cultures of different ages were used in the experiments.

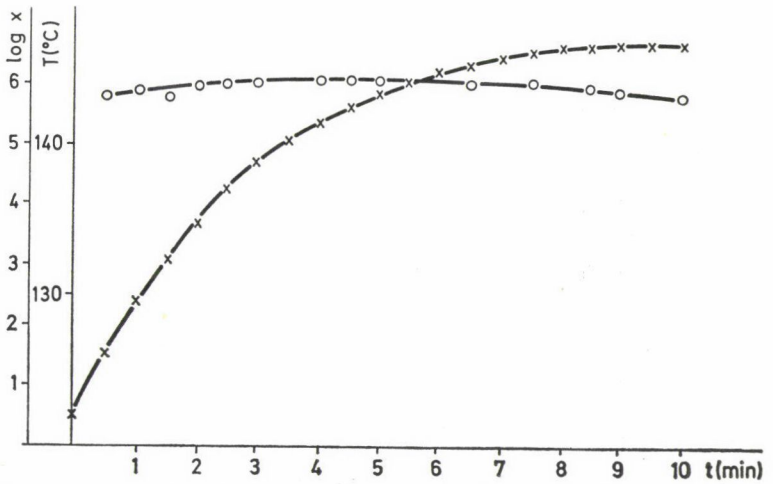

Fig. 10. Temperature (T) and viable cell count (x) changes in the course of heat treatment (t) of *B. stearothermophilus*. Medium: glycerol
— ○ — ○ — Viable cell count
— × — × — Temperature

Series 1 and 2: *B. stearothermophilus* in 4-day cultures; amount of sample: 0.2 ml suspension per glass tube.

Series 3 and 4: *B. stearothermophilus* in 40-day cultures; the amount of the sample in experiment 3 is 0.1, in experiment 4 0.2 ml per tube.

Temperatures and viable cell counts in Series 1 are given in Fig. 11, while those in Series 3 in Fig. 12. The results are summarized in Table 6.

Table 6

Results of thermal destruction experiments with B. stearothermophilus

1			2			3			4		
T_i (K)	D (min)	k (min^{-1})	T_i (K)	D (min)	k (min^{-1})	T_i (K)	D (min)	k (min^{-1})	T_i (K)	D (min)	k (min^{-1})
394	4.55	0.506	393.45	3.85	0.322	393.75	3.57	0.645	394.8	2.50	0.921
395.1	3.33	0.692	394.55	3.85	0.598	395.15	2.11	1.061	396.0	1.56	1.48
395.95	1.67	1.38	395.6	2.27	1.015	396.45	1.25	1.84	397.1	1.06	2.17
396.85	1.14	2.02	396.5	1.43	1.61	397.6	0.877	2.63	398.05	0.794	2.90
397.7	0.820	2.81	397.35	1.00	2.30	398.65	0.667	3.45	398.95	0.562	4.10
398.5	0.544	4.23	398.2	0.704	3.27	399.75	0.510	4.52	399.8	0.413	5.58
399.15	0.673	6.12	398.9	0.490	4.70	400.7	0.323	7.13			
			399.6	0.333	6.92						

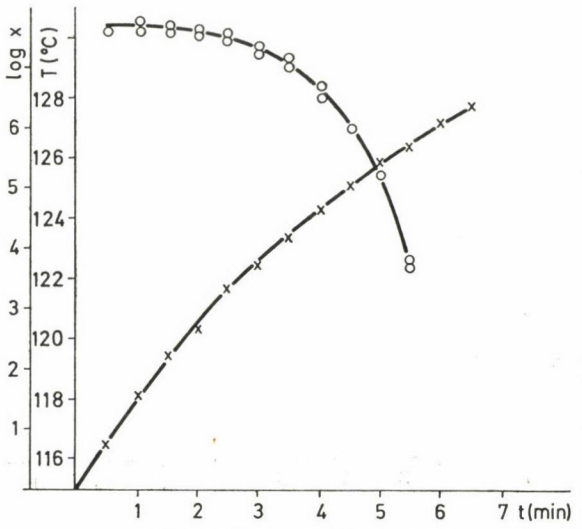

Fig. 11. Temperature (T) and viable cell count (x) changes as a function of treatment time (t) in experimental Series 1, *B. stearothermophilus*. Heat treatment medium: 0.5% glucose solution. Age of culture: 4 days

— ○ — ○ — Viable cell count
— × — × — Temperature

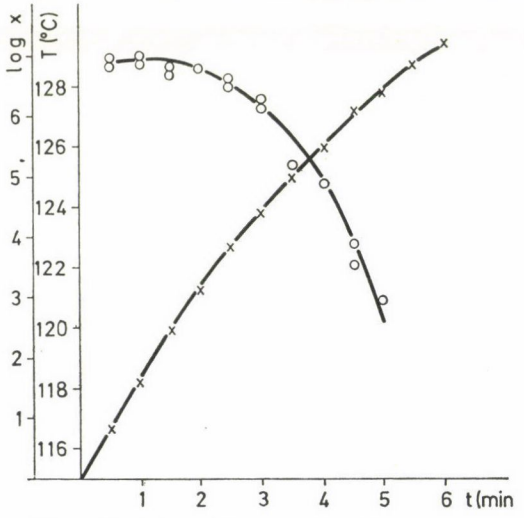

Fig. 12. Temperature (T) and viable cell count (x) changes as a function of treatment time (t) in experimental Series 3, *B. stearothermophilus*. Heat treatment medium: 0.5% glucose solution. Age of culture: 40 days

— ○ — ○ — Viable cell count
— × — × — Temperature

The equations belonging to the thermal death curves, the z values and the apparent activation energies are summed up in Table 7. Series 3 and 4 were evaluated separately because different amounts of suspension were used in these experiments.

The regression coefficients determined separately did not show any significant difference, the results of the two tests were therefore pooled and thus evaluated.

Table 7

Equation of thermal destruction curves, z values and apparent activation energies in B. stearothermophilus *experiments*

Series	Number of data pairs	Thermal destruction curve	r	z (K)	$z_{95\%}$ (K)	E (J mol^{-1})
Bac. st. 1, 2	15	$\lg D = 84.62 - 0.2130 \cdot T$ (393 K — 400 K)	—0.999	4.70	4.39—5.05	$(6.30 \pm 0.32) \cdot 10^5$
Bac. st. 3	7	$\lg D = 57.58 - 0.1449 \cdot T$ (393 K — 401 K)	—0.996	6.90	6.27—7.67	
Bac. st. 4	6	$\lg D = 61.25 - 0.1542 \cdot T$ (394 K — 400 K)	—0.999	6.49	5.56—7.78	
Bac. st. 3, 4	13	$\lg D = 58.85 - 0.1481 \cdot T$	—0.997	6.75	6.46—7.07	$(4.44 \pm 0.25) \cdot 10^5$

The thermal death curves are shown in Fig. 13, while the death rate coefficients against temperature in Fig. 14.

3. Conclusions

3.1. Evaluation of experiments with E. coli

On the basis of results as summarized in Table 3 no significant difference was found between the z values of cultures stored in three different ways. Similarly, no difference could be shown between apparent activation energies.

However, from the point of view of temperature resistance, different methods of storage and cultivation (as found on comparing D values belonging to identical temperatures) led to differences. These are shown in Fig. 4. The highest resistance was found in Series 2, cultivated at 310 K (37 °C) for 24 h. The next one was Series 1, cultivated at 310 K (37 °C) for 24 h and stored for 1 week at room temperature. The lowest resistance was observed with Series 3 and 4, grown at room temperature for 24 h.

The correlation between growth conditions and heat resistance as found in these experiments is in good agreement with the observations of Ellicker and Frazier (1938). These authors compared the heat resistance of cultures

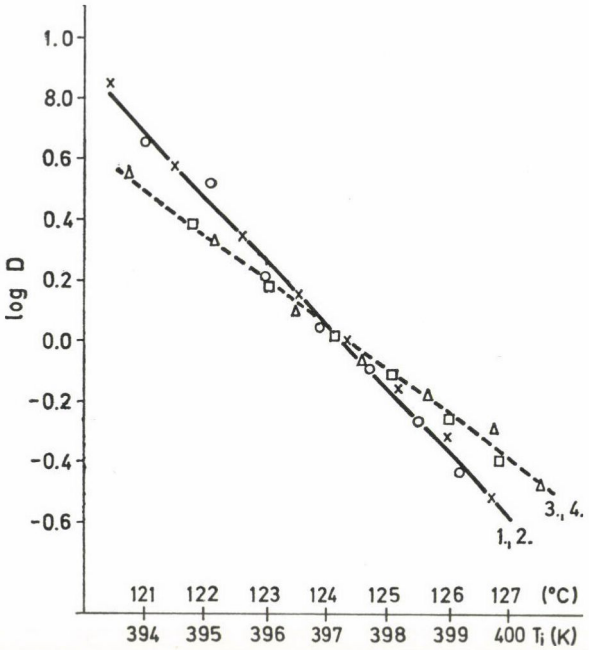

Fig. 13. Thermal death curves belonging to *B. stearothermophilus*, showing decimal reduction time (D) as a function of interpolated temperature (T_i). Curves 1 and 2: 4-day culture; Curves 3 and 4: 40-day cultures

— ○ — ○ — *B. stearothermophilus*, Series 1
— × — × — *B. stearothermophilus*, Series 2
— △ — △ — *B. stearothermophilus*, Series 3
— □ — □ — *B. stearothermophilus*, Series 4

grown at 38.5 or 40 °C with those grown at 28, 30 and 30.5 °C and found the lowest heat resistance in cultures grown at 28 °C.

The results obtained in Series 3 and 4 showed very good agreement, thus they could be combined and this proves the reproducibility of the new experimental method.

According to data in the literature (SKINNER & HUGO, 1976) the heat resistance of *E. coli* largely depends on the water activity of the medium and on its composition. GOEPFERT and co-workers (1957) found for instance that with $a_W = 0.99$ at 330.2 K (57.2 °C) the D value was 1.2 min, while with $a_W = 0.9$ it was 46.5 min.

CHAMBERS and co-workers (1957) found, in nutrient broth of 329 K (56 °C), a D value of 4.5 min and a z value of 4.9 K. According to LEMCKE and WHITE (1959) a decimal reduction time of 4 min was found at 328 (55 °C) in Ringer solution.

The water activity of the 0.5% glucose solution applied in these experiments was higher than 0.99. The data found in the literature closely approxi-

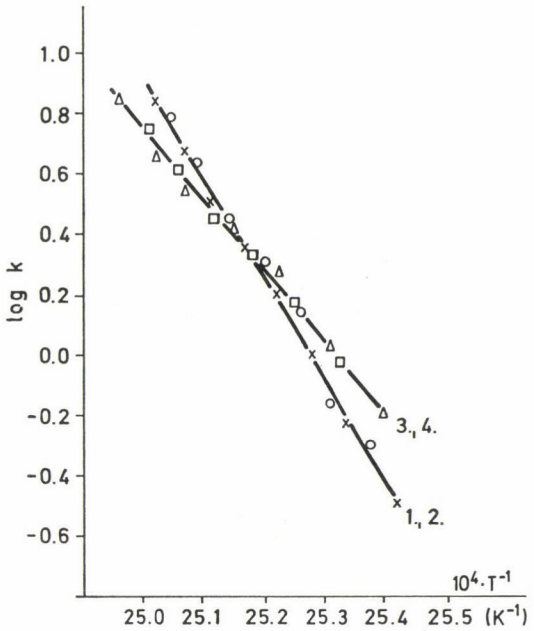

Fig. 14. Arrhenius plots of the death rate coefficients (k) as obtained as a function of interpolated temperature (T_i) in experiments with *B. stearothermophilus*. Curves 1 and 2: 4-day cultures; Curves 3 and 4: 40-day cultures

— ○ — ○ — *B. stearothermophilus*, Series 1
— × — × — *B. stearothermophilus*, Series 2
— △ — △ — *B. stearothermophilus*, Series 3
— □ — □ — *B. stearothermophilus*, Series 4

mate the results of Series 1 of *E. coli*. [Cultivating at 310 K (37 °C) for 24 h and storage for 1 week at room temperature.] The same experiment was carried out by the traditional method, too. The D values determined by the new method were calculated from the equation of the thermal death curve. The data in the literature referred to above and the results obtained in this experiment are given in Table 8.

Taking into account the great scatter of data found in the literature, a good agreement can be observed. The results of the new method fit particularly well those obtained by the traditional technique.

3.2. Evaluation of experiments with S. cerevisiae

On the basis of data summarized in Table 6 it may be said that the thermal death curve of the 24-h culture can be described by a straight line. The thermal death curve of the 168-h culture falls into two sections of significantly differing slope. Below 327 K (54 °C) the D value and the temperatures

Table 8

Comparison of thermal destruction data of E. coli

Temperature		Chambers *et al.* (1957)	Lemcke and White (1959)	Goepfert *et al.* (1970)	Present work	
(K)	(°C)				new method	traditional method
			D (minute)			
328	55		4		6.7	
329	56	4.5			3.52	3.54
330.2	57.2			1.2	1.6	
333	60				0.27	0.29
z (K)		4.9			3.58	3.68
95% confidence interval (K)					3.39—3.80	3.68

belonging to them fit well the data of the 24-h culture. The thermal death curve of the cultures of different age is described by a practically common regression equation. Above 327 K (54 °C) the slope of the thermal death curve is less steep, and is characterized by a higher *z* value. Accordingly, the apparent activation energy of the destruction process is lower than with temperatures below 327 K (54 °C) or with the 24-h culture in the whole temperature interval.

The results obtained with the new experimental method are well reproducible, as proven by the fitting data of parallel measurements.

The thermal death parameters as determined by the traditional technique and by the new method were compared to the data of Kiss and Clarke (1969). These authors studied the thermal death of *S. cerevisiae* strains grown at 302 K (29 °C) for 20 h. The data for comparison are given in Table 9. The data obtained by the new method were calculated from the equation of the thermal death curve.

As can be seen, the *D* values fit extremely well. Considering the confidence interval of the *z* value determined by Kiss and Clarke (1969), the difference in this respect was not significant either. The data obtained by the author of this study by the traditional technique and the new method fit particularly well.

3.3. Evaluation of experiments with B. stearothermophilus

3.3.1. Experiments in glycerol medium. Below 420 K (147 °C), no substantial death was observed. Results permit the conclusion that heat activation was of a low measure. The resistance to the high temperature was due to the fact that the applied medium, glycerol, contained very little water, below 10% and that, due to the very low water activity, the thermal resistance of the bacterial spores was higher than under moist conditions (Russel, 1971).

Table 9

Thermal destruction data of S. cerevisiae

Temperature		KISS and CLARKE (1969)		Present work	
				new method	traditional method
(K)	(°C)	*D* (min)			
318	45	335	385	380	
323	50	20.5	11.7	15.8	13.4
326	53			2.35	2.17
328	55	1.25	2.50	0.67	
329	56			0.35	0.38
z (K)		4.12*	4.57*	3.62	3.88
95% confidence interval (K)		3.54—5.56*		3.45—3.82	3.36—4.59

* Regression data calculated from the results of the authors.

3.3.2. Experiments in glucose solution. As can be seen in Table 7, there is a significant difference between the *z* values and the apparent activation energies of the 4-day and 40-day cultures. The *z* value of the 40-day culture is higher and thus its apparent activation energy is lower than that of the 4-day culture.

No difference was observed between the results of parallel measurements, the reproducibility of the method is thus very good. The different amounts of suspension introduced into the tubes in Series 3 and 4 did not affect the results. The thermal death curves obtained with tubes containing 0.1 and 0.2 ml suspension were practically identical whereby the use of a common equation was made possible as it is shown in Table 7. The difference between the amounts introduced into the tubes is of no consequence, because their time constant of heat transfer corresponds to that of the thermometer. Thus the temperature read on the thermometer is the same as in the tubes.

On comparing the combined results of experiments carried out on older cultures with those of COOK and GILBERT (1965) and of BRIGGS (1966), as measured in aqueous medium, the agreement was found very good. Data to be compared are given in Table 10.

Summing up, it may be said that the new method was equally suitable for the determination of thermal death data at temperatures below and above 373 K (100 °C). The results obtained by the new method are reproducible and agree very well with those found in the literature and obtained by the author using the traditional technique.

Table 10

Thermal destruction data of B. stearothermophilus

Temperature		Cook and Gilbert (1965)	Briggs (1966)	Present work
(K)	(°C)	*D* (min)		
388	115	25	24	24.4
391	118		8.4	8.8
394	121		4	3.15
z (K)			7	6.46–7.07

The new method is substantially more rapid and simpler than the techniques hitherto applied and it eliminates the sources of error inherent in them. Since it permits the modelling of the temperature profiles of sterilization procedures at varying temperatures, as is the case in practice, the method may be applied with a substantially higher degree of safety.

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DETERMINATION OF THE PROTEIN CONTENT OF CERTAIN MEAT PRODUCTS BY ULTRAVIOLET ABSORPTION SPECTROPHOTOMETRY

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The protein and fat content of meat products may be solubilized by preparing a suspension with sodium hydroxide solution from a known quantity of the appropriately minced and homogenized sample, and treating its aliquot part with acetic acid–chloroform mixture. Subsequent to the separation of non-soluble components by filtration, the solute may be photometrically studied at the wavelength corresponding to the absorption maximum. Percentual protein content may be determined from the obtained optical density by means of the calibration line or of the regression equation. This technique is rapid and of sufficient accuracy as compared to the *Kjeldahl* method and it is therefore suitable to on-line control, ready-product control and serial testing alike. The spectrophotometer is easy to handle and the technique is cheap in view of its low requirements for chemicals.

Proteins have characteristic absorption maxima in the ultraviolet range. The peptide bond leads to a stronger maximum between the wavelengths of 180–230 nm, while aromatic amino acids present in the proteins (tyrosine, tryptophan, phenyl-alanine) provide a smaller but typical absorbance between the wavelengths of 250–300 nm (SCHORMÜLLER, 1965).

The method developed to determine protein content in milk on this basis (NAKAI & LE, 1970), and the method elaborated for beef products, egg yolk and flour (TOMA & NAKAI, 1971) have proved that, after proper separation, the measurement of absorption in the ultraviolet range — being proportional to the protein content – may be applied for the determination of protein content in foodstuffs as well.

Our investigations were extended to determine the protein content in Hungarian salami, in sliced Italian sausage and in Bologna sausage. Samples were obtained from the meat packing plant at Szeged of the TRUST FOR LIVESTOCK TRADE AND MEAT INDUSTRY. Our aim was to enable this branch of industry to use the same technique and chemical reagents in the formulation of the analytical procedures and to adopt the same method for the determination of the protein content in various products.

1. Materials and methods

1.1. Mincing and homogenization of the samples

Samples are minced in a kitchen grinder two times. In case of the higher fat content of Hungarian salami and sliced Italian sausage a rest period of at least 1 h at 5–10 °C is necessary in order to obtain a more solid consistency of the fatty substance. This is how homogeneity may be secured for these products in the course of mincing.

Minced and homogenized samples are then stored in air-tight vessels at 5–10 °C.

1.2. Chemicals and materials

0.1 *N* sodium hydroxide solution,
97% acetic acid (v/v),
chloroform, anal. pure,
589[1] *Schleicher Schuell* quantitative filter paper.

1.3. Devices

UV Spectrophotometer,
1 cm silica cell,
KM–8 kitchen grinder,
Biomix homogenizer,
100 ml bulb pipette,
1 ml bulb pipette with double mark,
10 ml burette,
glass-stoppered test tube.

1.4. Process of determination

100 ml of sodium hydroxide, previously heated to 40 °C, is pipetted into the *Biomix* vessel to the homogenized sample weighed in with 0.01-g accuracy. The mixture is then left to rest in order to allow the protein fibres and other components to loosen. It is then homogenized in the *Biomix* homogenizer. One ml of the homogeneous suspension, obtained in this way, is immediately pipetted into a test tube and 7 ml of acetic acid, then 2 ml of chloroform are added from a burette. The solid components left over after mixing are removed by filtration from the solution. Absorbance is measured at 273 nm.

Composition of the photometric blank: 1.00 ml of sodium hydroxide, 7.00 ml of acetic acid and 2.00 ml of chloroform. (Similarly to the examined solution the solvent mixture should also be filtered.)

The wavelength of measurement was established from the above specified solution by way of taking the spectrum. For solutions of all the three products the absorption maximum occurred at 273 nm. Figure 1 illustrates the spectrum of the protein solution made of Bologna sausage.

Table 1

Production of alkaline suspensions of the different meat products

Meat product	Sample (g)	Rest period (min)	Homogenization period (min)
Hungarianla sami	3.00	15	15
Sliced Italian sausage	4.00	10	10
Bologna sausage	6.00	5	5

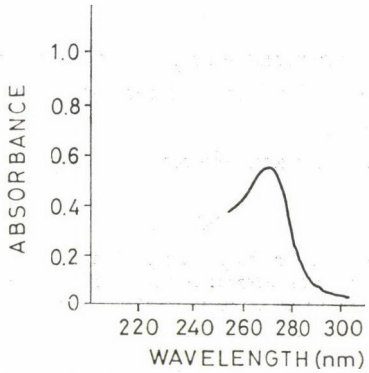

Fig. 1. Protein solution made of Bologna sausage

1.5. Allowance for disturbing conditions

From among the substances present in the products, the possible effects of fat on the results of spectrophotometry were investigated in such a way that in accordance with the routine test specified under section 1.4 the spectrum of pork fat was taken (0.2 mg fat was weighed to make 100 ml of suspension) in the wavelength range of 330–260 nm. The absorbance did not change and consequently the presence of fats does not affect or disturb the protein values obtained.

1.6. The plotting of the calibration line, regression equation

The optical density obtained by spectrophotometry should be compared with the percentual protein content obtained by the *Kjeldahl* method. To plot the line, samples with different protein contents (at least four) should be made

and homogenized by applying the original sample and other appropriate materials.

The measurements performed by us are summarized in Table 2.

Table 2

Preparation of samples with different protein contents for plotting the calibration line

Sample	Weight (g)							
	Hung. salami	Fat	Sliced Ital. sausage	Meat	Fat	Bologna sausage	Meat	Fat
1.	20.00	0.00	70.00	0.00	10.00	60.00	0.00	20.00
2.	15.00	5.00	40.00	40.00	0.00	70.00	0.00	10.00
3.	10.00	10.00	60.00	20.00	0.00	40.00	40.00	0.00
4.	5.00	15.00	20.00	60.00	0.00	60.00	20.00	0.00

The adequately homogenized samples are photometrically measured according to the routine test. Protein content determination by the *Kjeldahl* technique is also performed.

The average values of 5 parallels are shown in Table 3.

Table 3

Data necessary for the plotting of the calibration line in order to determine the protein content by spectrophotometry in certain meat products

Sample	Optical density	Protein content (% by *Kjeldahl*)
Hungarian salami		
1.	0.607	28.43
2.	0.512	23.67
3.	0.370	15.48
4.	0.227	8.22
Sliced Italian sausage		
1.	0.490	12.60
2.	0.693	19.62
3.	0.600	16.72
4.	0.775	22.46
Bologna sausage		
1.	0.628	12.58
2.	0.540	9.83
3.	0.810	15.93
4.	0.983	19.92

Under the same spectrophotometric conditions construction of a single calibration curve for each product suffices.

Figures 2, 3 and 4 show the calibration lines of Hungarian salami, sliced Italian sausage and Bologna sausage.

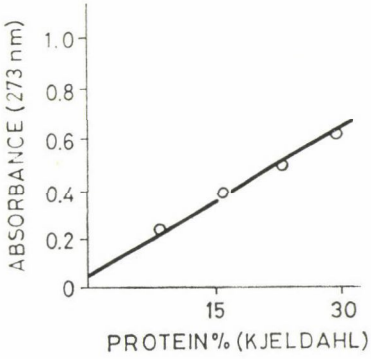

Fig. 2. Calibration line for salami

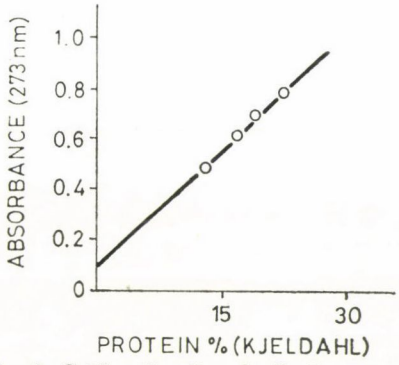

Fig. 3. Calibration line for Italian sausage

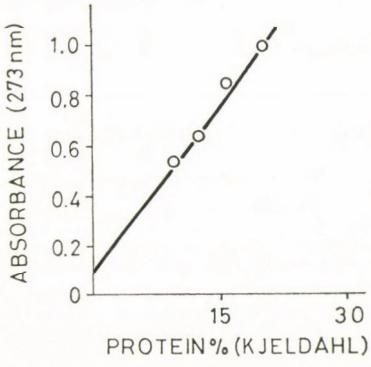

Fig. 4. Calibration line for Bologna sausage

The different slopes of the lines might possibly be explained by the different quantities of protein of the connective tissue of the three products (this is not measured by the spectrophotometric method) and by the varying amounts of foreign proteins (*e.g.* soy protein) which have no absorption maxima at the wavelength of 273 nm.

Regression lines were computed from data in Table 3. From the two regression curves the type

$$K = a + b\,A_{273}$$

is used which is obtained by linear regression calculations. In the equation A_{273} is the measured absorbance, K is the sought value of the *Kjeldahl* protein content, expressed in %. Exact values for a and b may be found under sections 2.1, 2.2 and 2.3.

1.7. Simplified calibration

When only the construction of the calibration line is required and no analysis of the accuracy of the method is needed, then the samples with different protein contents may be produced in a substantially simpler way.

Suspensions are made with different amounts of the examined meat product under the conditions prescribed in Table 1. Their absorbances are measured according to section 1.4.

For the construction of the calibrating line, the average percentual protein content of the original sample is still to be measured by the *Kjeldahl* procedure.

The line is plotted on the basis of the considerations here below.

The percentual protein content of the sample by the *Kjeldahl* technique corresponds to the optical density of the solution, which is prepared from the suspension as prescribed in Table 1. The percentual protein contents of the other suspensions are calculated from the measured optical densities by assuming direct proportionality. The corresponding data are plotted in a coordinate system.

Accordingly, the simplified calibration line of the sliced Italian sausage can be constructed from the data contained in Table 4.

The weighing of 4.00 g sample is prescribed for the routine test to prepare 100 ml of suspension.

The average protein content of the sample by *Kjeldahl* is 14.45% (Table 6).

Table 4

Data for construction of the simplified calibration line of sliced Italian sausage

Sample weighed into the suspension (g)	Optical density of the protein solution prepared from the suspension	Percentual protein content corresponding to the optical density
4.00	0.515	14.45*
3.50	0.475	12.64**
3.00	0.415	10.83**
2.50	0.380	9.02**
2.00	0.325	7.23**

* Measured
** Calculated

2. Results

For the mathematical investigation of the accuracy of the elaborated analytical procedures, protein contents of samples prepared in conformity with specifications under section 1.1 were determined by both the spectrophotometric process outlined in section 1.4 and by the *Kjeldahl* method; 15 parallels were analysed. By means of the relationships described under section 1.6, the percentual protein content values were calculated from the optical density values.

Data for the various products are contained in Tables 5, 6 and 7.

For the correlations shown under sections 2.1, 2.2 and 2.3, the t test provides a highly significant result (at the $P = 99\%$ probability level) to discard the null-hypothesis.

2.1. Mathematical analysis for the accuracy of protein determination by spectrophotometry in Hungarian salami

The regression equation is as follows:

$$K = -4.09722 + 53.72312\ A_{273}.$$

The value of the correlation coefficient is

$$r = 0.9996.$$

Table 5

Measurement of protein content in Hungarian salami by UV spectrophotometric and Kjeldahl *methods*

Serial number	Optical density	Calculated protein content %	*Kjeldahl* protein, content %
1.	0.615		28.42
2.	0.620		28.49
3.	0.597		28.43
4.	0.617		28.40
5.	0.603		28.40
6.	0.619		28.45
7.	0.597		28.43
8.	0.608		28.53
9.	0.609		28.53
10.	0.601		28.47
11.	0.607		28.41
12.	0.610		28.46
13.	0.603		28.51
14.	0.596		28.40
15.	0.614		28.53
Averages	0.608	28.55	28.46

It has been established that the *Kjeldahl* protein content in Table 5 was within the $P = 95\%$ confidence limit of the regression line. Significance of the correlation was tested by the t test (SVÁB, 1973).

For the average optical density of Table 5 the *Kjeldahl* value from the regression line is: 28.55%. The departure of the measured average from this is:

$$28.55 - 28.46 = 0.09 < ts_k \sqrt{\frac{1}{4} + \frac{(A^x_{273} - \bar{A}_{273})^2}{\Sigma' A^2_{273}}} =$$

$$= 4.3 \cdot 0.3 \sqrt{0.25 + \frac{(0.608 - 0.429)^2}{0.083}} = 1.03\%.$$

In the above formula, t is the tabulated value (for $P = 95\%$, number of degrees of freedom: 2), s_k is the mean scatter in *Kjeldahl* protein % made from deviations from the regression line, A^x_{273} is the average optical density from Table 5, $\bar{A}_{273}$ is the average optical density belonging to the regression line, $\Sigma' A^2_{273}$ is the squared sum of deviations from the mean of optical density values for the computation of the regression line.

Table 6

Measurement of protein content in sliced Italian sausage by UV spectrophotometric and Kjeldahl *methods*

Serial number	Optical density	Calculated protein content %	*Kjeldahl* protein content %
1.	0.520		14.73
2.	0.535		14.28
3.	0.525		14.30
4.	0.535		14.47
5.	0.532		14.25
6.	0.528		14.46
7.	0.520		14.51
8.	0.520		14.76
9.	0.522		14.68
10.	0.522		14.40
11.	0.520		14.35
12.	0.532		14.33
13.	0.537		14.41
14.	0.520		14.38
15.	0.538		14.45
Averages	0.527	13.99	14.45

2.2. Mathematical analysis for accuracy of protein determination in sliced Italian sausage by spectrophotometry

The regression equation can be described by the equation:

$$K = -4.09616 + 34.31768\ A_{273}.$$

The correlation coefficient is:

$$r = 0.9993.$$

The *Kjeldahl* protein content according to Table 6 was established to be within the $P = 95\%$ confidence limit of the regression line.

The significance of the correlation was tested as in 2.1 by the t test. For the average optical density of Table 6, the *Kjeldahl* value calculated from the regression line is: 13.99%. The deviation of the measured average from this is:

$$14.45 - 13.99 = 0.46 < 0.59.$$

Table 7

Measurement of protein content in Bologna sausage by UV spectrophotometric and Kjeldahl *methods*

Serial number	Optical density	Calculated protein content %	*Kjeldahl* protein content %
1.	0.690		14.43
2.	0.690		13.50
3.	0.700		13.57
4.	0.690		13.26
5.	0.680		13.16
6.	0.680		13.35
7.	0.680		13.20
8.	0.695		13.63
9.	0.685		13.34
10.	0.685		13.34
11.	0.680		13.19
12.	0.680		13.41
13.	0.690		13.20
14.	0.690		13.22
15.	0.690		13.35
Averages	0.687	13.39	13.34

2.3. Mathematical analysis for the accuracy of protein determination in Bologna sausage by spectrophotometry

The regression equation can be described by the equation:

$$K = -1.74144 + 22.02828\ A_{273}.$$

The correlation coefficient is:

$$r = 0.9967.$$

The *Kjeldahl* protein content according to Table 7 was established to be within the $P = 95\%$ confidence limits of the regression line.

The significance of the correlation was tested as in 2.1 by the t test. For the mean optical density in Table 7, the *Kjeldahl* value calculated from the regression line, is: 13.39%. The deviation of the measured average from this is:

$$13.39 - 13.34 = 0.05 < 0.44\%.$$

3. Conclusions

Mathematical calculations indicate that spectrophotometric methods in the UV range developed for the determination of protein content are of sufficient accuracy in comparison to the *Kjeldahl* method.

Their practical introduction is primarily advisable in areas where a large number of rapid measurements is required.

This technique is rapid in comparison to the *Kjeldahl* method: given the calibration line (under the same photometric conditions, this line should be established only once for a given product), the time necessary to obtain one datum is about 40 minutes, but this time is appreciably shorter in case of serial analyses.

The spectrophotometer is easy to handle and it requires no special skills or expertise.

Chemicals are used in only small amounts.

The technique may be equally introduced in case of on-line control or for ready-product analysis and quality control outside the plant.

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CHARACTERIZATION OF COW'S MILK AND BUFFALO MILK BY POLYACRYL-AMIDE GEL ELECTROPHORESIS

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Attempts were made to find out whether cow's milk and buffalo milk may be distinguished by polyacryl-amide gel electrophoresis of their proteins. The effect of different heat treatments (at 85 and 120 °C) on these proteins was also investigated.

Results indicate that buffalo milk contains a higher amount of proteins than cow's milk and it possesses a rapidly migrating casein fraction that can not be detected in cow's milk.

As regards whey proteins, buffalo milk is also quantitatively richer than cow's milk and there is a relative departure in the quantities of various fractions as well. These features provide a possibility to distinguish between the two kinds of milk and their various mixtures.

Subsequent to heat treatment the amount of whey proteins decreases in the milk of both kinds of animal but to a higher extent in buffalo milk. The quantity of casein proteins tends to increase as a consequence of heat treatment, proportionately to the fall in whey proteins; this is explained by the fact that, upon heat treatment, the protective colloid system of caseins diminishes and, by the formation of a complex with whey proteins, some fractions are formed which migrate together with casein proteins.

Milk as a foodstuff with nutritionally valuable animal protein content plays a significant role in ensuring adequate protein supply for the ever increasing human population.

For reasons associated with tradition and as a result of the general farming pattern in Hungary and in other European countries primarily, or almost exclusively, only cow's milk is today commercialized and consumed. However, in a number of developing countries, milk from other animals is also used. In Vietnam about half the cattle stock is represented by buffaloes; buffalo milk is therefore a major factor in the milk supply for the population.

Several papers are available on studies into the physical, chemical and biological characteristics of the milk of the cow and/or of the buffalo and on changes occurring during processing of these milks.

In practice, however, a separate collection and processing of the two kinds of milk is not possible. Consequently, it has become necessary to study phenomena which arise after mixing these two kinds of milk or which are expected to occur in the course of processing the mixture. There are several lines of investigations under way at our Department.

In this paper we review the results obtained in our investigations into the qualitative and quantitative properties of milk proteins, in particular the possibility of using polyacryl-amide gel electrophoresis, as a tool of analysis, to characterize the proteins and the changes occurring in the milk mixtures.

1. Materials and methods

The milk used for the analysis was obtained from the cow (breed: Hungarian speckled) and the buffalo, both in the 5th months of their lactation period, of a farmer around Kaposvár. The two kinds of milk were mixed in the following proportions: 75 : 25%, 50 : 50%, 25 : 75%. Thus, including the two original milks, 5 samples were obtained altogether.

The milk samples were heat-treated under laboratory conditions at two temperature values widely applied in practice; pasteurization at 85 °C and sterilization at 120 °C.

The protein content in the milk samples was determined with the *Pro Milk* apparatus. The theoretical principle of the method was the well-known capacity of protein to bind a dye (*Amidoblack* 10 B) whereby a complex compound is formed as a precipitate. After removal of the precipitate, inference can be made to the protein content from the drop in concentration of the colorant in the solution (UZONYI, 1971; UZONYI & MOLNÁR, 1974). In the process of analysis the amount of total protein, casein protein and whey protein was determined.

The preparation of milk samples for polyacryl-amide gel electrophoretic analysis was based on a procedure which has been tested many times (PHAM VAN MINH & KÁDAS, 1978). In order to separate casein and whey proteins, the pH value of defatted milk was adjusted to 4.5 with 1 N acetic acid under constant agitation; the precipitated casein proteins were then separated by centrifuging from the filtrate containing the whey proteins. The precipitate was solubilized by means of the electrode buffer of 4.5 M related to urea (8.3 pH Tris-glycine buffer).

Electrophoresis for the monomer was performed in a 7.5% acryl-amide gel. For casein 20 μl, for whey protein 40 μl, protein-containing solution was applied to the gel columns and a current intensity of 2 mA per tube was used in the course of electrophoresis. At a temperature of 8 °C the electrophoresis lasted between 3.5–4 hours, depending on the number of tubes in operation. Subsequent to electrophoresis, the gels were dyed by *Amidoblack* solution in order to render the protein fractions visible. The *Amidoblack* was removed from the non-adsorbing part (devoid of protein) by means of multiple washing with a 7% acetic acid solution. The quantitative evaluation of the protein fractions separated in the gels was performed by a *Chromoscan*-type densitometer.

2. Results and conclusions

The results of the quantitative protein determinations carried out with the *Pro Milk* apparatus are given in Table 1. In case of untreated milk samples these data reflect sufficiently well the fact that the total protein content of buffalo milk is higher than that of cow's milk and that the level of casein and whey proteins also exceeds that in cow's milk. For the various milk mixtures the situation is featured by the tendency that with an increasing proportion of buffalo milk increases the amount of proteins in the mixtures and this tendency holds for all three kinds of protein under study.

Practically there is no alteration in the total protein content of the various milk samples which could be ascribed to the effect of heat treatment at either pasteurization or sterilization temperatures; but there is an appreciable shift in the proportion of casein and whey proteins – the amount of whey proteins decreases and that of casein proteins increases. This peculiarity becomes more and more distinct with the rise of temperature. The data also indicate that whey proteins in buffalo milk are more sensitive to heat than those in cow's milk. This is shown by the fact that in untreated samples the whey protein content is higher with a higher proportion of buffalo milk, there is no appreciable change in pasteurized mixtures while it decreases in the above sequence in sterilized samples.

The above outlined alterations in the amount of casein and whey proteins upon heat treatment may be adequately explained by the investigations of WHITE and SWEETSUR (1977). According to these, the protective colloids are weakened by the higher temperature, which leads to easier complex formation and precipitation between whey and casein proteins.

On the basis of the results of protein determinations with the *Pro Milk* apparatus, we tried to decide whether the application of polyacryl-amide gel electrophoresis was suitable to distinguish between the milks of the cow and of the buffalo and to describe the quantitative changes occurring in the course of heat treatment.

There are certain considerations which have to be kept in mind in the evaluation of analysis by polyacryl-amide gel electrophoresis. The number of proteins occurring in whole milk is relatively high; it may amount to 30–40. Accordingly, a great number of bands may be observed on the electropherograms, although in practice the above indicated value is never reached; in addition, there are large deviations in values disclosed in different papers. This may be explained by the differences in methodology, sample preparation, by divergent traits of the breeds giving the milk and other important factors. In this study by the separation of casein and whey proteins on the electropherograms the number of fractions has been considerably reduced. On the other hand, not aiming at preparative separation, the fractions were grouped

and their changes characterized. It should also be kept in mind that in case of heat-treated milks the quantitative results obtained by gel electrophoresis do not always correspond to the protein determinations obtained by other methods, or they may only be compared to the latter after appropriate correction. The differences arise from the fact that under the effect of heat treatment protein

Table 1

Protein content and protein distribution in different mixtures of cow's milk and buffalo milk
(Mean value and standard deviation in the case of 4 parallel analyses)

Milk sample	Total protein %	Casein protein %	Whey protein %
Untreated (normal) milk			
cow's milk	3.61 ± 0.42	2.99 ± 0.10	0.61 ± 0.57
75% cow's milk – 25% buffalo milk	3.70 ± 0.17	3.02 ± 0.32	0.68 ± 0.26
50% cow's milk – 50% buffalo milk	3.86 ± 0.64	3.12 ± 0.30	0.74 ± 0.24
25% cow's milk – 75% buffalo milk	4.02 ± 0.51	3.23 ± 0.52	0.79 ± 0.33
buffalo milk	4.08 ± 0.38	3.24 ± 0.16	0.84 ± 0.41
Pasteurized milk (85 °C)			
cow's milk	3.58 ± 0.10	3.13 ± 0.70	0.45 ± 0.62
75% cow's milk – 25% buffalo milk	3.65 ± 0.52	3.17 ± 0.38	0.48 ± 0.48
50% cow's milk – 50% buffalo milk	3.80 ± 0.16	3.31 ± 0.46	0.49 ± 0.28
25% cow's milk – 75% buffalo milk	4.01 ± 0.41	3.52 ± 0.45	0.49 ± 0.19
buffalo milk	4.07 ± 0.46	3.64 ± 0.12	0.43 ± 0.36
Sterilized milk (120 °C)			
cow's milk	3.55 ± 0.71	3.34 ± 0.35	0.21 ± 0.63
75% cow's milk – 25% buffalo milk	3.58 ± 0.08	3.43 ± 0.42	0.15 ± 0.38
50% cow's milk – 50% buffalo milk	3.81 ± 0.35	3.69 ± 0.50	0.12 ± 0.36
25% cow's milk – 75% buffalo milk	4.02 ± 0.40	3.89 ± 0.11	0.13 ± 0.48
buffalo milk	4.08 ± 0.33	3.98 ± 0.36	0.10 ± 0.50

aggregates may also be formed which in the course of the given electrophoretic technique, which depends on pore size of the gel, may not be forced to migrate. They are thus retained in the zone of application on the gel. Densitograms prepared from the electropherograms of casein proteins from treated and untreated cow's and buffalo milk are illustrated in Fig. 1. The quantitative results are compiled in Table 2. Simple visual inspection indicates – and this is also confirmed by numerical data – that buffalo milk is richer in casein than cow's milk and that all the analogous protein fractions exceed the corresponding

ones in cow's milk. In addition, buffalo milk contains an extremely mobile casein fraction which is not observed in cow's milk. Thus this latter feature presents the basis for distinguishing between the two kinds of milk.

These properties are characteristic of heat-treated milk as well. The quantitative increase in casein proteins as a result of heat treatment can be

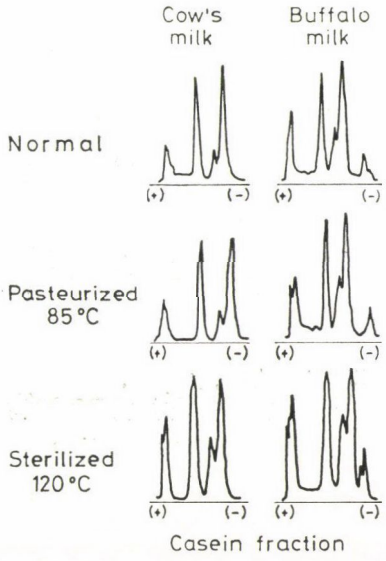

Fig. 1. Densitograms made from electropherograms of casein proteins in cow's milk and buffalo milk

Table 2

Analysis of casein protein by gel electrophoresis in individual milk samples
[Evaluation by densitometer, on the basis of the size (T) and percentage distribution (%) of the peak area]

Milk sample	Fractions										ΣT = 100%
	A		B		C		D		E		
	T	%	T	%	T	%	T	%	T	%	
	Cow's milk										
Untreated	3.5	15.4	7.2	31.7	2.5	11.0	9.5	41.9	—	—	22.7
Pasteurized (85 °C)	4.0	17.0	6.8	29.0	2.3	9.8	10.4	44.2	—	—	23.5
Sterilized (120 °C)	6.5	19.1	12.0	35.3	4.5	13.2	11.0	32.4	—	—	34.0
	Buffalo milk										
Untreated	6.0	18.5	8.2	25.3	4.5	13.9	10.2	31.5	3.5	10.8	32.4
Pasteurized (85 °C)	6.2	18.6	7.5	22.5	5.7	17.1	11.0	33.1	2.9	8.7	33.3
Sterilized (120 °C)	7.2	17.8	10.4	25.6	6.1	15.0	13.6	33.5	3.3	8.1	40.6

observed in both kinds of milk; the extent of this increase is smaller at pasteurization and greater at sterilization temperature.

Results of investigations of whey proteins are illustrated in Fig. 2 and Table 3. Untreated buffalo milk is richer in whey proteins than cow's milk. The qualitative analysis of whey proteins shows that the amount of low

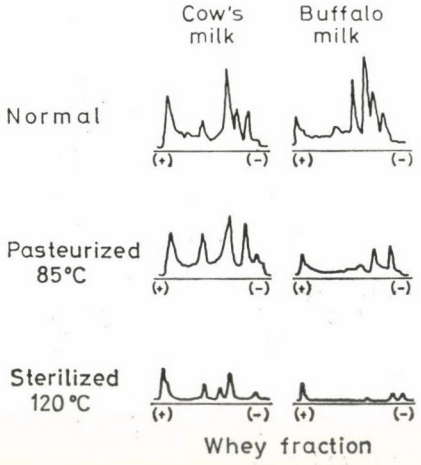

Fig. 2. Densitograms made from electropherograms of whey proteins in cow's milk and buffalo milk

Table 3

Analysis of whey proteins by gel electrophoresis in individual milk samples
[Evaluation by densitometer, on the basis of the size (T) and percentage distribution (%) of the peak area]

Milk sample	Fractions												ΣT = 100%
	A		B		C		D		E		F		
	T	%	T	%	T	%	T	%	T	%	T	%	
	Cow's milk												
Untreated	5.2	25.4	3.5	17.1	6.1	29.7	2.5	12.2	3.2	15.6	—	—	20.5
Pasteurized (85 °C)	4.6	24.0	4.0	20.8	6.2	32.3	2.3	12.0	2.1	10.9	—	—	19.2
Sterilized (120 °C)	2.1	31.3	0.8	12.0	1.3	19.4	1.7	25.3	0.8	12.0	—	—	6.7
	Buffalo milk												
Untreated	3.4	14.9	2.2	9.6	4.8	21.1	5.4	23.7	4.4	19.3	2.6	11.4	22.8
Pasteurized (85 °C)	2.8	28.6	—	—	2.0	20.4	2.4	24.5	—	—	2.6	26.5	9.8
Sterilized (120 °C)	1.2	50.0	—	—	0.2	8.4	—	—	0.5	20.8	0.5	20.8	2.4

mobility fractions is relatively greater in cow's milk, while in buffalo milk more mobile fractions prevail.

The results adequately support the fact that the amount of whey proteins is reduced by heat treatment in the milk of both animals. Whey proteins in buffalo milk are substantially more sensitive to heat; certain fractions are completely missing in their electropherograms and after sterilization the amount of whey proteins is practically an order of magnitude lower than in untreated milk.

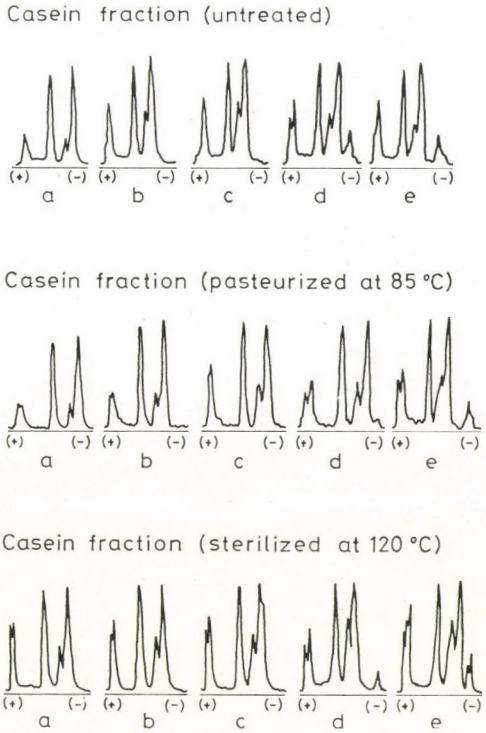

Fig. 3. Densitograms made from electropherograms of casein proteins in the mixture of cow's milk and buffalo milk

These results are in good agreement with findings on cow's milk obtained in other investigations (PHAM VAN MINH *et al.*, 1978).

Figure 3 and Table 4 illustrate the qualitative and quantitative characteristics of the casein content of milk mixtures and the changes caused by heat treatment. In the untreated milk mixtures, increasing the proportion of buffalo milk increases the amount of casein proteins. In samples where the proportion of buffalo milk predominates, the high mobility protein fraction characteristic of buffalo milk becomes apparent as it is also indicated by the Figures.

Table 4

Analysis of casein proteins by gel electrophoresis in mixed milk samples
[Evaluation by densitometer, on the basis of the size (T) and percentage distribution (%) of the peak area]

Milk sample	Fractions										ΣT = 100%
	A		B		C		D		E		
	T	%	T	%	T	%	T	%	T	%	
	Untreated milk										
cow's milk	3.5	15.4	7.2	31.7	2.5	11.0	9.5	41.9	—	—	22.7
75% cow's milk – 25% buffalo milk	4.6	16.4	8.0	28.6	3.6	12.9	11.8	42.1	—	—	28.0
50% cow's milk – 50% buffalo milk	6.1	19.2	9.2	28.9	4.5	14.2	12.0	37.7	—	—	31.8
25% cow's milk – 75% buffalo milk	6.0	18.5	8.1	25.0	4.2	13.0	12.0	37.0	2.1	6.5	32.4
buffalo milk	6.0	18.5	8.2	25.3	4.5	13.9	10.2	31.5	3.5	10.8	32.4
	Pasteurized milk (85 °C)										
cow's milk	4.0	17.0	6.8	29.0	2.3	9.8	10.4	44.2	—	—	23.5
75% cow's milk – 25% buffalo milk	5.2	18.7	8.1	29.0	3.8	13.6	10.8	38.7	—	—	27.9
50% cow's milk – 50% buffalo milk	6.1	19.9	7.9	25.7	4.6	15.0	12.1	39.4	—	—	30.7
25% cow's milk – 75% buffalo milk	6.3	18.3	8.5	24.7	5.4	15.7	11.6	33.7	2.6	7.6	34.4
buffalo milk	6.2	18.6	7.5	22.5	5.7	17.1	11.0	33.1	2.9	8.7	33.3
	Sterilized milk (120 °C)										
cow's milk	6.5	19.1	12.0	35.3	4.5	13.2	11.0	32.4	—	—	34.0
75% cow's milk – 25% buffalo milk	6.8	19.5	11.7	33.5	4.6	13.2	11.8	33.8	—	—	34.9
50% cow's milk – 50% buffalo milk	7.1	19.8	10.5	29.2	5.7	15.9	12.6	35.1	—	—	35.9
25% cow's milk – 75% buffalo milk	6.8	17.7	9.8	25.5	5.9	15.3	13.8	35.8	2.2	5.7	38.5
buffalo milk	7.2	17.8	10.4	25.6	6.1	15.0	13.6	33.5	3.3	8.1	40.6

The quantitative increase in casein proteins due to heat treatment occurs in milk mixtures as well. The ratio of single fractions related to the total casein content does not depart significantly from that observed in untreated milk mixtures.

As far as changes in their qualitative or quantitative properties are concerned, the whey proteins of milk mixtures do not display any course similar to that shown by casein proteins (Fig. 4 and Table 5). Some generally valid

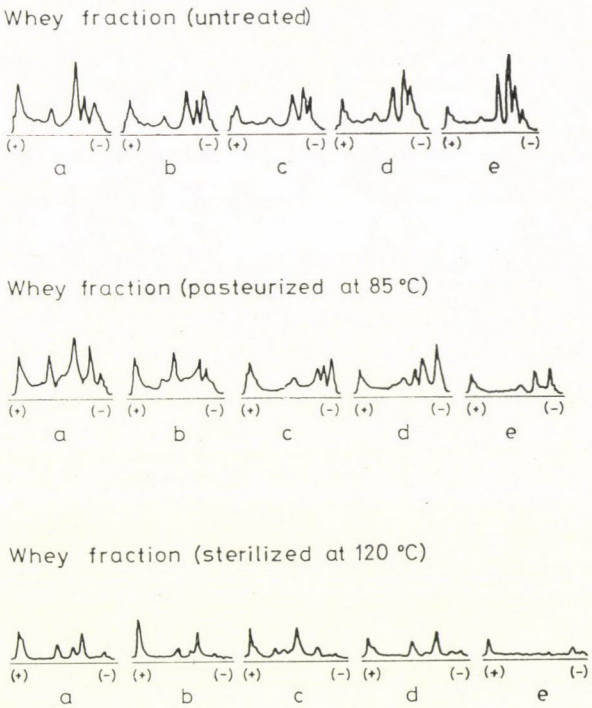

Fig. 4. Densitograms made from electropherograms of whey proteins in the mixtures of cow's milk and buffalo milk

characteristics may also be found for whey proteins; thus, in comparing untreated milk samples the amount of the less mobile fraction with low migration speed may be observed to decrease with the rise in the proportion of buffalo milk. In the course of heat treatment the amount of whey proteins diminishes inversely with increasing temperature. It emerges also from the investigations of milk mixtures that the heat sensitivity of whey proteins in buffalo milk is considerably higher than in cow's milk, thus in a mixture where buffalo milk is present in a larger proportion, an increased quantitative degradation of whey proteins may be observed at identical heat treatment.

Table 5

Investigation of whey proteins by gel electrophoresis in case of milk mixtures
[Evaluation by densitometer based on the size (T) and the percentage distribution (%) of the peak area]

Milk sample	Fractions												ΣT = 100%
	A		B		C		D		E		F		
	T	%	T	%	T	%	T	%	T	%	T	%	
	Untreated milk												
cow's milk	5.2	25.4	3.5	17.1	6.1	29·7	2.5	12.2	3.2	15.6	—	—	20.5
75% cow's milk – 25% buffalo milk	4.5	24.7	2.9	15.9	4.6	25.3	2.4	13.2	3.8	20.9	—	—	18.2
50% cow's milk – 50% buffalo milk	4.0	23.1	2.6	15.0	4.3	24.9	3.1	17.9	3.3	19.1	—	—	17.3
25% cow's milk – 75% buffalo milk	4.2	19.4	2.8	13.0	4.2	19.4	4.4	20.4	4.6	21.3	1.4	6.5	21.6
buffalo milk	3.4	14.9	2.2	9.6	4.8	21.9	5.4	23.7	4.4	19.3	2.6	11.4	22.8
	Pasteurized milk (85 °C)												
cow's milk	4.6	24.0	4.0	20.8	6.2	32.3	2.3	12.0	2.1	10.9	—	—	19.2
75% cow's milk – 25% buffalo milk	4.5	25.4	4.1	23.2	4.2	23.7	4.9	27.7	—	—	—	—	17.7
50% cow's milk – 50% buffalo milk	4.3	25.2	2.8	16.4	3.2	18.7	3.1	18.1	3.7	21.6	—	—	17.1
25% cow's milk – 75% buffalo milk	3.2	19.3	2.8	16.8	3.0	18.1	3.4	20.5	4.2	25.3	—	—	16.6
buffalo milk	2.8	28.6	—	—	2.0	20.4	2.4	24.5	—	—	2.6	26.5	9.8
	Sterilized milk (120 °C)												
cow's milk	2.1	31.3	0.8	12.0	1.3	19.4	1.7	25.3	0.8	12.0	—	—	6.7
75% cow's milk – 25% buffalo milk	2.1	38.9	0.6	11.1	0.7	13.0	1.6	29.6	0.4	7.4	—	—	5.4
50% cow's milk – 50% buffalo milk	1.8	32.2	0.6	10.7	0.6	10.7	1.8	32.2	0.6	10.7	0.2	3.5	5.6
25% cow's milk – 75% buffalo milk	1.6	29.6	0.9	16.7	0.4	7.4	1.7	31.5	0.4	7.4	0.4	7.4	5.4
buffalo milk	1.2	50.0	—	—	0.2	8.4	—	—	0.5	20.8	0.5	20.8	2.4

The above investigations show that polyacryl-amide gel electrophoresis is an appropriate tool to distinguish buffalo milk from cow's milk and mixtures of the two kinds of milk. This is due to the difference which can be observed in the number of fractions in casein proteins as well as the differences in the sensitivity of their whey proteins to heat.

*

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OCCURRENCE OF 3,4-BENZPYRENE IN FATS AND HEAT-INDUCED CHANGES IN ITS CONCENTRATION

K. Soós

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Carcinogenic and co-carcinogenic polyaromatic hydrocarbons (PAH) may contaminate foodstuffs, including edible oils. Representing the carcinogenic effect, 3,4-benzpyrene serves as an indicator of the contamination by other PAH compounds. Hungarian data have not been available so far and data in the literature were contradictory as to the effect of heat treatment on these compounds.

It was found that, in the edible oils, margarines and hardened oils studied, 3,4-benzpyrene occurred in the range of 1–11 $\mu g\ kg^{-1}$, a level higher than permissible from the view-point of hygiene. This necessitates the continuous control of 3,4-benzpyrene contamination in vegetable oils and the revision of the vegetable oil manufacturing technology.

In the course of laboratory frying tests as well as in sunflower seed and rape-seed oil samples taken in commercial fish and dough frying shops it was proved without any doubt that heating of these oils to 200–300 °C reduced their 3,4-benzpyrene content.

When heated to the relatively low temperature of 120 °C, the 3,4-benzpyrene content of the oil may increase when, for instance with potatoes, benzpyrene is introduced to the system without being counteracted by the rate of degradation.

This does not permit the conclusion that oils may be heated infinitely. Apart from a number of unfavourable changes this is automatically limited by the formation of acrolein, itself toxic and mutagenic.

Many of the representatives of polyaromatic hydrocarbons belong to the group of carcinogenic compounds the carcinogenic effect of which has been proven not only in test animals but also in human beings. A number of statistical data verify the carcinogenic effect of polyaromatic hydrocarbons occurring in foodstuffs, *e.g.* the data demonstrating a direct relationship between consumption of strongly smoked meat and cancer of the stomach (SHABAD, 1961; DUNGAL & SIGURJONSSON, 1967). Similar observations were made in Hungary: a correlation was found between the higher-than-the-average mortality due to cancer of the stomach of the Wends in Western Hungary and their consumption of smoked meat highly contaminated by polyaromatic hydrocarbons (SOÓS & HAJDU, 1974).

In the survey of PAH contamination in foods, vegetable oils and fats are of considerable importance, particularly in relation to 3,4-benzpyrene. Since 3,4-benzpyrene occurs frequently in foods it serves as an indicator of the presence of other carcinogenic PAH compounds.

The PAH contamination in vegetable oils may originate from several sources. The plant may absorb these compounds from waste gases in the air

and the drying and roasting of oil seeds may enhance their amount in the seeds. The solvents used to extract, after pressing, the residual oil from the seeds might also constitute an important source of contamination. The benzene distillates used are frequently contaminated with PAH compounds (HOWARD *et al.*, 1968). The heat exchangers or other equipment, if damaged, may also permit the infiltration of these contaminants.

In Hungary the PAH contamination of vegetable oils was first studied by PERÉDI and RUZICS (1976), however, their investigations were extended to the separation and quantitative assessment of the PAH groups, only.

The aim of this study was to demonstrate the extent of contamination with 3,4-benzpyrene in Hungarian vegetable oils and fats and further to establish the changes caused in their benzpyrene content by heating. Data found in the literature are inconsistent. HUTCHISON and ALEXANDER (1963) observed the formation of cyclic compounds when linseed oil was heated. According to ZALDIVAR (1959) the consumption of over-heated fats caused cancer of the stomach in test animals. At the same time BORNEFF and FABIAN (1966) and later FRITZ (1968) proved that the heating of fats and oils at 320 °C did not lead to the formation of PAH compounds, on the contrary, due to thermic degradation, the amount of these substances decreased during heating.

It is well known that during heating of fats and oils in the presence of oxygen unfavourable changes occur, shown also by Hungarian investigations (ÁLDOR, 1963). One of the most unfavourable changes is the formation of acrolein of toxic and mutagenic effect (LÜCK & SOUCI, 1958). In the course of frying experiments carried out under industrial conditions, changes in the 3,4-benzpyrene content and the formation of acrolein were studied.

1. Materials and methods

1.1. Materials

1.1.1. Fat samples. The sunflower seed oil, rape-seed oil, margarine and domestic lard samples were taken from the market, the fats hardened from oil were obtained by the courtesy of the VEGETABLE OIL FACTORY, Rákospalota. Samples were taken and analysed in 1976.

1.1.2. Fat samples used for frying under laboratory conditions. Sunflower seed and rape-seed oils were heated for 10 h on an electric heater. After taking samples, the heating of the oils was continued for another 10 h. The temperature of the oils was maintained between 270 and 300 °C.

For frying potatoes, 5 kg were peeled, sliced and fried continuously in about 600 g sunflower seed oil. Frying required about 10 h. The temperature varied between 110 and 130 °C.

1.1.3. Fat samples used for frying under industrial conditions. Samples were taken in fish and dough frying shops prior to the beginning of frying and after frying when the oil was considered by the staff as "tired" and was replaced by fresh oil. In two dough frying shops and a fish frying shop rape-seed oil and in one dough frying shop the 1 : 1 mixture of rape-seed oil and lard have been used.

1.2. Methods

1.2.1. Determination of the 3,4-benzpyrene content. Together with other polyaromatic hydrocarbons, 3,4-benzpyrene was extracted with nitromethane by the method developed in the INSTITUTE OF NUTRITION, based on the method of FRITZ (1976).

Hundred g of edible oil or of other fats were dissolved in 200 ml cyclohexane and transferred to a 500-ml separating funnel. The solution was shaken with 5×50 ml freshly distilled nitromethane 5 min each time. The combined nitromethane extracts were placed in a covered beaker and kept in a deep freezer (−18 °C) for at least 12 h. The frozen supernatant oil and some cyclohexane were filtered in the freezer through a folded filter and the latter was rinsed with a small amount of precooled nitromethane. (The frozen oil thaws in about 2–3 min, thus the operation has to be carried out at −18 °C.) The nitromethane solution was evaporated just to dryness in a rotating vacuum evaporator and the residue was dissolved in a few ml of cyclohexane.

Subsequently, the clean-up of the extract by preparative thin-layer chromatography, the separation of the 3,4-benzpyrene-containing fraction and the determination of 3,4-benzpyrene by UV spectrophotometry and spectrofluorescence were carried out by the method developed earlier for the analysis of smoked foods (Soós, 1976). The UV spectra were taken with an *Unicam* SP-8000 apparatus and the fluorescence spectra were evaluated directly from the acetylated cellulose thin-layer by means of a *Farrand* UV-VIS-2 instrument ("in situ" technique). The absolute sensitivity of the method is 1 ng 3,4-benzpyrene, and the relative sensitivity under the prevailing experimental conditions is 0.1 μg kg^{-1}. The average deviation between parallel measurements was $\pm 20\%$.

1.2.2. Determination of the acrolein content. Acrolein formed and accumulated during heating of the fat was determined by the spectrophotometric method of ROSENTHALER and VEGEZZI (1954) using the resorcin colour reaction. The acrolein was extracted from 5 g oil with 5 ml 96% ethanol by shaking for 2 min. Subsequently the method described above was applied. The absolute sensitivity of the method is 50 μg acrolein, while the relative sensitivity under the experimental conditions is 10 mg kg^{-1}.

2. Results

The 3,4-benzpyrene content of the fat samples is given in Table 1. The results were compared to data in the literature, which are also presented. It should be mentioned here that SIEGFRIED (1975) and FRITZ (1976) used also nitromethane to extract the PAH compounds, the method of HOWARD and co-workers, however, essentially differs from ours, as they used dimethylsulfoxide for extraction. As regards sensitivity of the 3,4-benzpyrene determination there was practically no difference between the above-mentioned three methods and the one applied in this study.

Table 1

The 3,4-benzpyrene content of fats used in Hungary as compared with data in the literature
Period of sampling: January and February, 1976
Period of analysis: First quarter of 1976

Product	Number of samples	3,4-benzpyrene content ($\mu g\ kg^{-1}$)		References
		average	range	
Sunflower seed oil	4	3.7	3.0— 5.0	present study
Rape-seed oil	4	5.7	1.5—11.8	present study
Margarine, LIGA	2	3.0	2.5— 3.5	present study
Margarine, RAMA	2	6.5	5.5— 7.5	present study
Hardened oil	2	8.2	7.5— 8.9	present study
Domestic lard	2	ND	ND—ND	present study
Vegetable oils and margarines in the FRG	5	2.6	<0.2—5.2	SIEGFRIED, 1975
Edible oils in the GDR	10	1.0	0.1— 1.5	FRITZ, 1976
Olive oil, USA	5	0.5	0.4— 0.5	HOWARD *et al.*, 1966

ND = Not detectable, less than 0.1 $\mu g\ kg^{-1}$.

The 3,4-benzpyrene content of sunflower seed and rape-seed oils maintained at high temperature is illustrated in Fig. 1.

The conditions applied in potato frying were described in detail in para. 1.1.2. In the experiments carried out in 1976 the initial 3,4-benzpyrene content of the sunflower seed oil was 0.27 $\mu g\ kg^{-1}$. After the continuous frying of 5 kg potatoes the 3,4-benzpyrene content of the oil increased to 0.42 $\mu g\ kg^{-1}$. The 3,4-benzpyrene content of the peeled raw potatoes, used in the experiment was 0.2 $\mu g\ kg^{-1}$.

Data on oil samples taken in dough and fish frying shops are given in Table 2.

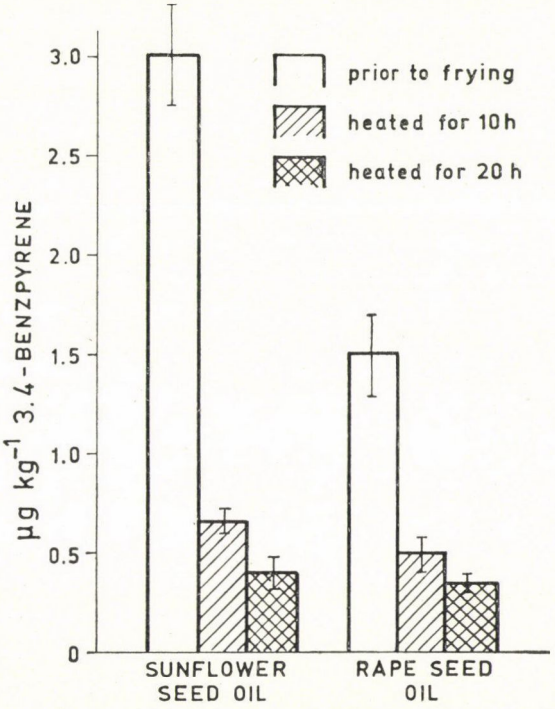

Fig. 1. Reduction of the 3,4-benzpyrene content of edible oils during frying under laboratory conditions. Frying temperature: 270–300 °C

Table 2

Decrease in the 3,4-benzpyrene content of edible oils as influenced by continued heating in fish and dough frying shops
Sampling and analysis: March, 1977
Place of sampling: Budapest, in co-operation with the Station for Public Hygiene and Epidemiology (Budapest)

Frying shop	Type of oil	Heating period (h)	Temperature (°C)	3,4-benzpyrene ($\mu g\ kg^{-1}$)	Acrolein ($mg\ kg^{-1}$)
Dough frying shop I	rape-seed	—	—	26.7	—
	oil	3.5	205—210	10.2	141
Dough frying shop II	rape-seed	—	—	8.1	—
	oil	4.0	220	4.1	128
Dough frying shop III	50% rape-seed oil	—	—	5.7	—
	50% lard	15	210	3.3	118
		27	210	3.6	80
Fish frying shop	rape-seed	—	—	8.4	—
	oil	3.0	170	7.5	64

3. Conclusions

As indicated above (Table 1) in the investigated edible oils and margarines prepared from hardened oil and in fats, 3,4-benzpyrene was found to be present in the range from 1 to 11 $\mu g\ kg^{-1}$. This is problematic from the view-point of hygiene. The results were higher than those found in similar products in the GDR and the USA, and even surpassed the relatively high values obtained in the FRG. These facts necessitate the continuous control of 3,4-benzpyrene in vegetable oils and in accordance with the findings, the revision of the technology. Each phase of the technological process has to be investigated in order to establish where the bulk of the contamination enters the product.

According to BRAMMER (1973) the majority of the carcinogenic polyaromatic substances is removed from the oil in the course of refining, thus in our cases the possibility of a subsequent contamination has to be taken into account.

Table 1 also shows that, in domestic lard, 3,4-benzpyrene has not been detected. This supports the observations found in the literature according to which the PAH compounds are metabolized relatively rapidly in the animal organism and do not accumulate (GORELOVA *et al.*, 1970).

Heating experiments in the laboratory have shown a substantial reduction in the 3,4-benzpyrene content of oils at temperatures above 270 °C. The reduction is rapid in the first 10 h of heating and slows down between the 10th and 20th h. This phenomenon is due to the thermal degradation of benzpyrene. In the course of this process the rings split and, due to the action of heat and the oxygen content of the air, various oxidation products are formed (BORNEFF & FABIAN, 1966). These experiments support the findings of BORNEFF and FABIAN (1966) and those of FRITZ (1968) as well who also observed the reduction of the PAH content as a consequence of the heating of vegetable oils.

The observation that the 3,4-benzpyrene content of fats used in the dough and fish frying shops also decreases is satisfying from the view-point of hygiene. This was observed in spite of the fact that in this case the oil and the food form a complex system. A correlation has been established between the reduction of the benzpyrene content and the frying temperature. At the temperature applied in fish frying, *i.e.* at 170 °C the decrease has been less than at a dough frying temperature of 205–220 °C (Frying shops I and II), although the frying period in all the three shops varied between 3 and 4 h, the difference was not essential.

However, as may be seen in Table 1, a substantial amount of acrolein was formed in the oils during heating. The toxic and mutagenic effects of this compound are well known. In addition to other disadvantageous changes (increase in the acid and peroxide number, browning and deposition of combus-

tion products, etc.) this is a major argument for not permitting unlimited heating of oils. However, among the arguments the formation of carcinogenic polyaromatic substances does not figure.

Another result deserving attention is the fact that, in the course of frying of potatoes, the original 0.27 μg kg^{-1} 3,4-benzpyrene content of the sunflower seed oil increased to 0.42 μg kg^{-1}. The frying temperature of 110–130 °C is the consequence of the continuous heat removing effect of the evaporation of water from the potatoes. The slight increase in the benzpyrene content of the oil may be explained by the continuous introduction of benzpyrene with the potatoes (3,4-benzpyrene contamination in raw potatoes amounts to 0.2 μg kg^{-1}). At this relatively low temperature the rate of benzpyrene introduction is not counteracted by the rate of degradation.

*

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DETERMINATION OF Cl, K, Rb, Zn, Se AND Hg IN PAPRIKA BY NEUTRON ACTIVATION ANALYSIS

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(Received July 14, 1978; accepted December 12, 1978)

Various samples of Hungarian spice paprika (powdered) were analysed for their Cl, K, Rb and Zn content. Samples of paprika both with and without seeds were studied. The values are reported in ppm (dry weight).

Statistical analysis showed significant differences in the trace element contents between most cultivars. In the majority of the cases, increase in the seed content of the samples tended to reduce their trace element content except for Zn.

Toxic substances in the environment can be categorized as (i) naturally occurring toxic elements and compounds and (ii) toxic compounds that are synthesized industrially. The danger associated with naturally occurring toxic elements and compounds depends on their distribution in the environment. Under natural conditions their distribution remains relatively constant, largely because of natural biological processes that affect both their degradation and synthesis, and they do not pose serious public health problems. When used in industrial processes, however, they may re-enter the environment and disrupt the natural action of organisms in such a way that the balance between degradation can no longer be maintained (WOOD, 1974).

Several trace elements are considered essential, non-essential or toxic for animal life. However, all elements in high concentrations may become detrimental to organisms (SCHWARZ, 1972).

The nutritional role and the essentiality of trace elements as well as their biochemical and pathological significance to man and animals have been recently reviewed by several investigators (BOWEN, 1966; PARIZEK, 1972; UNDERWOOD, 1971).

As part of an attempt to increase the understanding of the distribution of elements both geographically and throughout the food chain (KARIMIAN-TEHERANI *et al.*, 1975) we measured some elements in Hungarian paprika.

1. Materials and methods

The powdered paprika samples were supplied by the PAPRIKA RESEARCH STATION, Kalocsa, Hungary (Table 1).

All analyses were made by comparing the spectra to those of known purity (pro anal.) and concentration (MERCK CHEMICAL Co.). Samples were

Table 1

Paprika cultivars studied in this report

No.	Samples	
1	K-D-601	determined growth type, not hot, without seeds
2	K-D-601	determined growth type, not hot, with 20% seeds
3	K-D-621	determined growth type, hot, without seeds
4	K-D-621	determined growth type, hot, with 20% seeds
5	K-M-622	half determined growth type, not hot, without seeds
6	K-M-622	half determined growth type, not hot, with 20% seeds
7	K-504	continuous growth type, not hot, without seeds
8	K-504	continuous growth type, not hot, with 20% seeds
9	K-V-1	continuous growth type, hot, without seeds
10	K-V-1	continuous growth type, hot, with 20% seeds

The red pepper (*Capsicum annuum*) cultivars were grown in the vicinity of Kalocsa (K) in Hungary

measured with a *Canberra* 1000-channel pulse height analyser connected to a 40 cm^3 Ge/Li detector.

Long half-life elements. – Samples weighing from 0.2 to 0.4 were sealed in quartz ampoules. The samples were irradiated for 24 hours at a thermal neutron flux of $7 \cdot 10^{13}$ n $cm^{-2}s^{-1}$. After a waiting period of 4 weeks, the samples were counted.

Short half-life elements. – Samples weighing from 0.08 to 0.15 g were sealed in PVC ampoules and irradiated for 5 minutes at a thermal neutron flux of $7 \cdot 10^{13}$ n $cm^{-2}s^{-1}$.

After waiting 1 hour the samples were counted.

2. Results

Figure 1 is a reproduction of a typical spectrum obtained after the neutron activation of paprika. The peaks measured were: ^{38}Cl 1642 keV, ^{42}K 1525 keV, ^{86}Rb 1080 keV and ^{65}Zn 1115 keV.

The mean values and standard deviations of the trace or minor element content in different samples can be found in Table 2 showing the chlorine, potassium, rubidium and zinc content of the examined paprika samples (see also Figs. 2–5). The significance of differences between samples with and without seeds was calculated using the *t* test (SACHS, 1972).

As can be seen, the potassium content is much higher than that of the trace metals (from 19444 to 32049 ppm wet weight). GIRARDI *et al.* (1967) for example, have measured the potassium content of powdered kale leaves from 24050 to 24800 ppm.

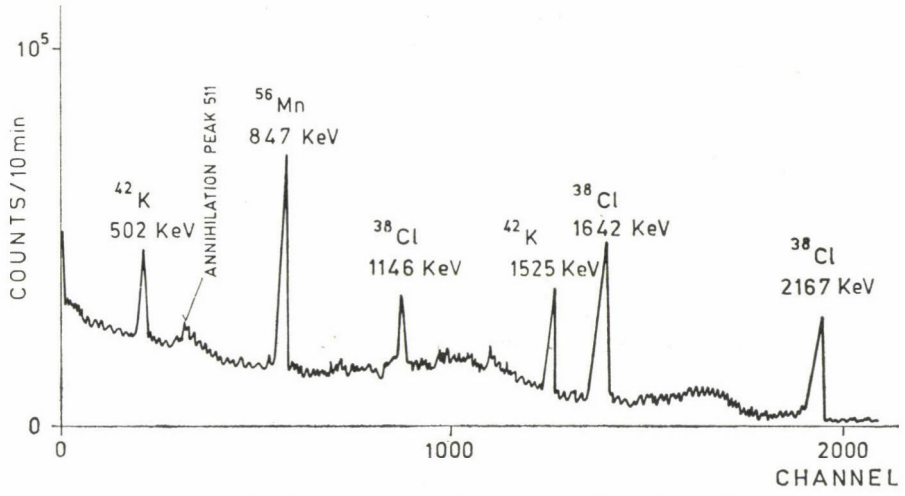

Fig. 1. Typical spectrum of neutron-activated paprika

Table 2

Average value ($\bar{x}$ ppm in the dry matter) and standard deviation (s, $n = 5$) of the chlorine, potassium rubidium and zinc contents in powdered paprika samples

No. of sample	Cl		K		Rb		Zn	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
1	3069	174	26 376	1630	13	1.67	16	1.3
2	2608	365	22 806	912	6	1.51	21	1.5
3	2707	474	32 049	1415	20	1.34	15	0.8
4	1800	281	30 980	1336	21	1.94	19	1.3
5	3276	201	27 697	803	9	1.81	16	1.3
6	3849	128	26 431	1146	12	1.34	26	2.0
7	3084	195	26 010	1430	10	1.81	17	1.2
8	2460	63	24 349	1135	12	1.22	29	1.4
9	3056	135	22 406	232	39	2.34	16	1.2
10	2291	248	19 444	338	31	5.27	21	1.1

BOWEN (1967) reported the chlorine, potassium and zinc content in tomato as 70, 7000 and 56 ppm with seed, compared to 25, 4700 and 21 ppm without seeds.

We also have found a different trace element content between paprika with and without seed.

The data for zinc from 15 to 29 ppm, chlorine from 1800 to 3849 ppm and rubidium from 6 to 39 ppm in Table 2 show a good agreement for the values recently reported in literature. There are even some measurements on feeding stuffs which indicate chlorine contents from 1800 to 2800 ppm, rubidium con-

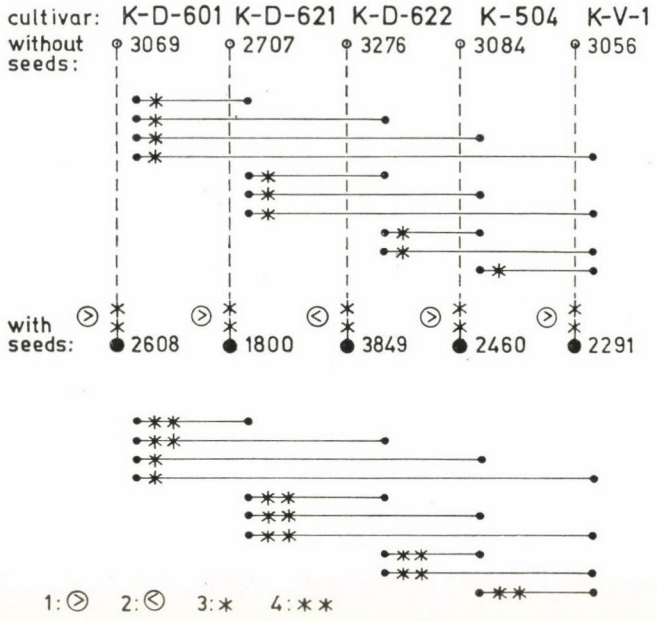

Fig. 2. Significance of differences in the chlorine content of powdered paprika samples, calculated with the t test (Figure: average values in ppm in the dry matter) 1: Cl content of sample without seeds higher than that with seeds; 2: Cl content of sample without seeds lower than with seeds; 3: difference not significant ($P > 0.05$)

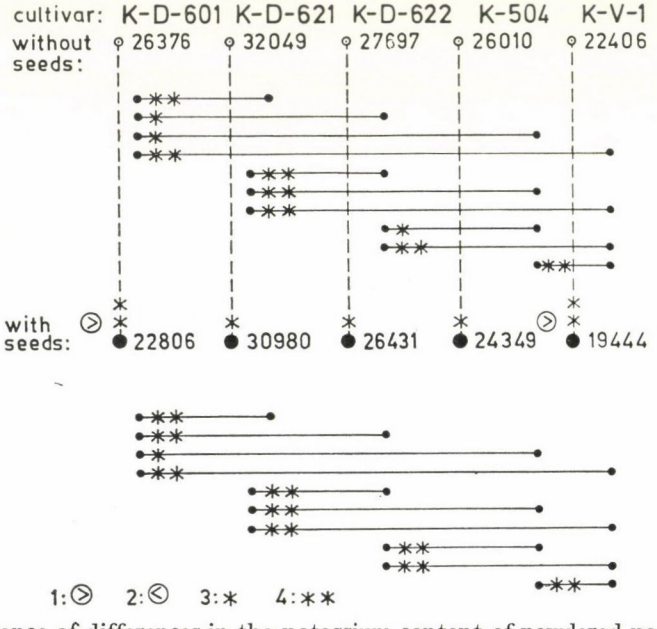

Fig. 3. Significance of differences in the potassium content of powdered paprika samples calculated using the t test (Figure: average values in ppm in the dry matter) 1: K content of sample without seeds higher than that with seeds; 2: K content of sample without seeds lower than that with seeds; 3: difference not significant ($P > 0.05$); 4: difference significant ($P \leq 0.05$)

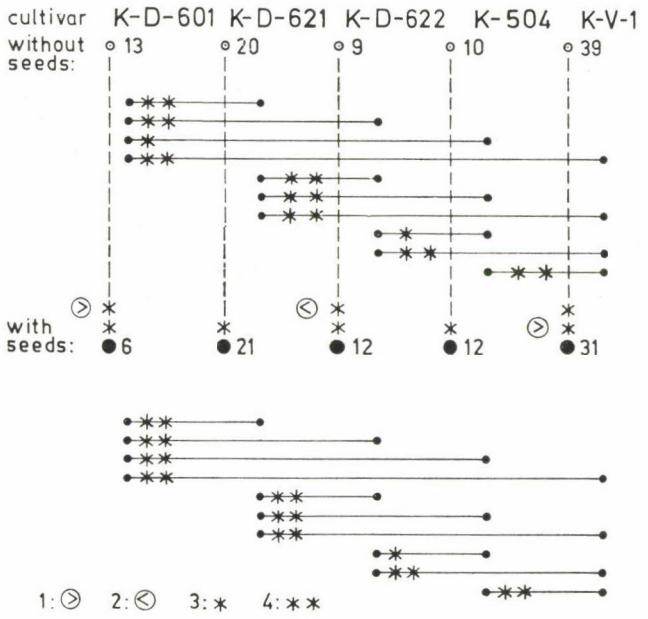

Fig. 4. Significance of differences in the rubidium content of powdered paprika samples, calculated using the t test (Figure: average values in ppm in the dry matter) 1: Rb content of sample without seeds higher than that with seeds; 3: difference not significant ($P > 0.05$); 4: difference significant ($P \leq 0.05$)

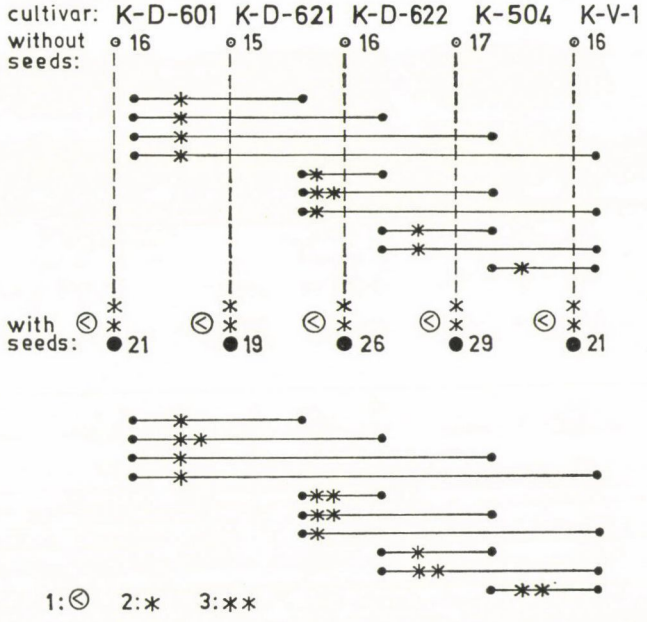

Fig. 5. Significance of differences in the zinc content of powdered paprika samples calculated with the t test (Figure: average values in ppm in the dry matter). 1: Zn content of sample without seeds higher than that with seeds; 3: difference not significant ($P > 0.05$); difference significant ($P \leq 0.05$)

tents from 3.5 to 73.0 ppm (BANCHER *et al.*, 1975; BECKER, 1974) and a Zn content in sugar beet leaves as 22 ppm (MORRISON, 1967).

In none of these samples was the concentration of Hg and Se beyond the threshold level of 0.04 ppm.

3. Conclusions

In the paprika samples ground with or without seeds there is a significant difference in the amounts of the elements studied.

The significance of differences is of various extent. It cannot be concluded definitely whether the enrichment of the elements occurs in the pod tissue of the fruit or in the seeds, although, in the majority of the cases, the trace element content of the samples without seeds appeared to be significantly higher than that of the samples containing 20% seeds (except Zn and two cases of Rb). The Zn content with seeds appeared to be significantly higher than that of the samples containing no added seeds. This was true for all four elements with cultivar K–504, for three elements (Cl, K and Zn) with cultivars K–D–601, K–V–1, K–D–622 and K–D–621 (Table 3).

The differences in Rb content of all cultivars with seeds and without seeds were not significant (Fig. 4).

In none of the samples was the concentration of Hg and Se beyond the threshold level of 0.04 ppm.

Table 3

Effect of increased seed content of ground paprika (5 cultivars) on the changes in their contents of 4 trace elements

Trace element	Cultivar					No. of cultivars in the "higher", "lower" or "not differing" categories			Total
	K-D-601	K-D-621	K-D-622	K-504	K-V-1	>	<	—	
Zn	<	<	<	<	<	0	5	0	5
Rb	>	<	<	<	>	2	3	0	5
Cl	>	>	<	>	>	4	1	0	5
K	>	—	—	—	>	2	0	3	5
No. of trace elements in the "higher", >	3	1	0	1	3	8			
"lower" or <	1	2	3	2	1		9		
"not differing" categories —	0	1	1	1	0			3	
Total	4	4	4	4	4				20

>: indicates higher trace element content in samples without seeds
<: indicate lower trace element content in samples without seeds
—: indicates no statistically significant difference

It would be useful to extend the experiments from the point of view of plant and human physiology. In order to get a closer view of the background it is necessary to examine the soil, agrotechnics, various parts and organs of the plant yield, as well as the industrial processing of paprika, and the accumulation of the elements in the human organism.

*

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CYTOGENETIC STUDIES WITH IRRADIATED GROUND PAPRIKA AS EVALUATED BY THE MICRONUCLEUS TEST IN MICE

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(Received August 9, 1978; accepted November 19, 1978)

Ground paprika irradiated with 3 Mrad proved to be non-clastogenic by the micronucleus test in mice fed paprika on 20% dry weight level of the semi-synthetic diet. Irradiated paprika does not influence the cell proliferative activity of the bone marrow according to the P/N ratio.

Spices have been, traditionally, used to impart characteristic flavour to foods in various parts of the world. The rapid advances in food industry and a wide acceptance of processed foods particularly in the affluent countries have remarkably increased demand for flavouring substances (CLARK, 1970). Spices in their natural state, therefore have become an important food commodity in the international market (GOTTSCHALK, 1977). Spices have, however, been shown to be microbially contaminated or prone to insect infestation and have been reported to cause spoilage of foods to which they are added (HADLOK, 1969; SCHÖNBERG, 1952; CHRISTENSEN *et al.*, 1967). In attempts to develop sterilization procedures, gamma radiation has been applied to sterilize spices with success (FARKAS, 1972; TJYBERG *et al.*, 1972; INAL *et al.*, 1975). Paprika is one of the important spices which can be disinfested by gamma radiation (40–70 krad) or can be preserved by a radurization (0.4–0.5 Mrad) process (FARKAS, 1975; FARKAS *et al.*, 1973). The feeding studies with diets containing irradiated paprika have also shown no deleterious effects in rats with regard to growth, clinical chemistry, reproduction, gross pathology (BARNA, 1973, 1974, 1976). In view of the expanding requirements of wholesomeness testing of irradiated foods for genetic effects (JOINT FAO/IAEA/WHO EXPERT COMMITTEE, 1969; CHAUHAN, 1974), mutagenicity tests were carried out with untreated, heat treated and irradiated (5 Mrad) paprika using *in vitro* (repair capacity of *Salmonella typhimurium* TA 1535, TA 98, TA 1975) and *in vivo* (HMAT with *Salmonella typhimurium* TA 1530) methods. The experiments indicated no mutagenic effect of paprika untreated, heat or radiation treated (CENTRAL FOOD RESEARCH INSTITUTE, Budapest, 1977). Present investigation was undertaken to examine the potential chromosomal damaging effect of irradiated paprika in the bone marrow of mice by using the micronucleus test (SCHMID, 1973).

1. Materials and methods

1.1. Animals

20 inbred Swiss female mice of about 10 weeks, obtained from the animal house of the BHABHA ATOMIC RESEARCH CENTRE, Bombay were randomized into four groups on the basis of weight and parentage and caged individually. Animals were allowed for two days to condition on the experimental room of the animal house and maintained at standard conditions.

1.2. Irradiation

Paprika (Hungarian standard commercial quality) was irradiated at a dose of 3 Mrad in a *Gamma Cell* 220 (ATOMIC ENERGY OF CANADA LTD.) at a dose rate of 5.2 krad min^{-1}. Irradiated paprika was fed in diets between 8 to 18 days after irradiation.

1.3. Diets and feeding

Two groups were continued on a stock ration and the other two were given test diets containing irradiated or unirradiated paprika (capsaicin-free, commercial quality from KALOCSAI PAPRIKA- ÉS KONZERVIPARI VÁLLALAT, Kalocsa) at 20% (dry weight) level and the feeding continued for 12 days. The composition of the stock ration and the test diets is given in Table 1.

Table 1

Composition of diets

	Stock ration (%)	Test diet (%)
Wheat	70.0	56.0
Bengal gram *(Cicer arietinum)*	20.0	16.0
Paprika (irradiated or unirradiated)	—	20.0
Dry yeast	4.0	3.2
Fish meal	5.0	4.0
Shark liver oil	0.25	0.20
Til oil	0.75	0.60

Wheat and Bengal gram were cracked before mixing with other dietary ingredients. The stock ration had about 8.7% moisture, 16.0% protein, 4.9% fat, 3.4% crude fibre and 3.2% ash. Test diet had about 8.6% moisture, 15.1% protein, 5.6% fat, 5.3% crude fibre and 3.6% ash.

Animals were observed every day for any untoward sign of behaviour, apparent toxicity and water intake. Daily food intake was measured for individual animals. Animals were weighed on the first and on the last day of the feeding period.

One of the groups fed on stock ration was given 100 mg kg^{-1} of hycanthone methane sulfonate 30 and 6 hrs before killing and served as a positive control.

1.4. Preparation of bone marrow smears

After a feeding period of 12 days, animals were killed by decapitation and both femur bones were excised and marrow smears were prepared as reported earlier (CHAUBEY *et al.*, 1975). Slides were stained in *May-Gruenwald, Giemsa* and mounted in Euparal. The slides were coded and two slides per animal were screened at 1000× magnification for the presence of micronucleated erythrocytes. 1000 polychromatic erythrocytes were scored per slide for the presence of micronuclei. The percentage frequency of the erythrocytes with micronuclei and the ratio of polychromatic to normochromatic cells were calculated per animal before computing the mean values for different groups.

1.5. Statistical evaluation

Data were evaluated by analysis of variance and *t*-test statistics (MORRISON, 1967). The *Bartlett* test was applied to determine the homogeneity of the variance. The difference was considered significant at $P \leq 0.05$.

2. Results and conclusions

The data on food intake and body weight did not show any significant differences among different groups (Table 2).

Table 2

Body weight and food intake

Group	Initial body weight g	Final body weight g	Food intake g/rat/day
Stock ration	16.5	16.4	22.5
Unirradiated paprika diet	16.7	16.7	22.1
Irradiated paprika diet	17.1	16.9	23.1
Hycanthone (Positive control)	17.0	17.0	21.0

As compared with other groups the positive control group given hycanthone showed a highly significant increase ($P < 0.001$) in the frequency of erythrocytes with micronuclei and a profound suppression of the cell proliferative activity as revealed by the decrease of P/N ratio (Table 3). This effects confirm our earlier observation in the highly clastogenic nature of hycanthone (CHAUBEY *et al.*, 1975).

Table 3

Incidence of erythrocytes with micronuclei in the bone marrow of mice

Group	Poly-E with micronuclei (%)	Normo-E with micronuclei (%)	Poly-E + Normo-E with micronuclei (%)	Poly-E / Normo-E
Stock ration	0.11 ± 0.06	0.06 ± 0.05	0.18 ± 0.11	1.02
Unirradiated paprika diet	0.23 ± 0.08	0.03 ± 0.05	0.27 ± 0.09	0.99
Irradiated paprika diet	0.11 ± 0.04	0.06 ± 0.02	0.17 ± 0.06	1.00
Hycanthone (Positive control)	4.5* ± 0.73	0.23* ± 0.08	4.74* ± 0.75	0.54*

± = Standard deviation; Poly-E = Polychromatic erythrocytes; Normo-E = Normochromatic erythrocytes

* $P < 0.001$ as compared with other groups, 5 animals were used in each group and 2 slides were prepared from each animal. Around 1000 polychromatic and the corresponding normochromatic erythrocytes were screened for presence of micronuclei per slide. Hycanthone (100 mg/kg) was injected intraperitoneally 30 and 6 hrs before killing the animals

The average incidence of micronuclei in the poly- and normochromatic erythrocytes were comparable among the groups given stock ration and the test diets containing irradiated or unirradiated paprika (Table 3).

The results of this study show no evidence of any clastogenicity in the bone marrow of mice that could be attributed to ingestion of irradiated paprika. The lack of any effect on the P/N ratio suggested that feeding of irradiated paprika does not influence the cell proliferative activity of the bone marrow.

*

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BOOK REVIEWS

Table grapes and refrigeration

Meeting of Commission C2 of the International Institute of Refrigeration and Commissions I. and III. of the International Vine and Wine Office, Paris, 12–14 October 1977.

The book contains 28 reports that were presented by experts from 14 countries on the following topics: Product characteristics (6 reports); Pre-harvest processes (6 reports); Processes between harvest and storage (3 reports); Storage and transport (13 reports).

Of the results reported, the following items merit special attention.

The best method to indicate the degree of maturity is to determine the sugar content of the grapes. The analytical method proposed is rapid, accurate and simple.

Application of the growth regulating substance gibberelline (GA3), renders the bunches looser, the grapes larger, stalks and stems thicker. The keeping time of the grapes increases.

During phases of growth and maturation water requirement of the plant culminates. Both the volume of the grapes and the bunch size may be increased by irrigation. It also has undesirable effects, such as delay in ripening and enhancement of the propagation of parasites.

Precooled and sulphured table-grapes packed in polythene remain fresh longer than refrigerated ones. Provided the grapes before packaging were kept relatively intact and without infection, this method preserves their freshness for at least 2 months even without refrigeration.

Conditions of the preservation of good grape quality: In the period from harvest to chilling: 27 °C, 20% relative humidity, 1 h. Cooling: 0 °C, 95% rel. humidity, 6 h. During storage: 0 °C, 95% rel. humidity, 7 days. Transport: 0–4 °C, 7 days.

The protopectin content of well-keeping grape cultivars remains high during storage. Varieties tolerating storage badly might be characterized by rapid changes in the protopectin fraction of the soluble pectin content.

Low pressure storage reduces weight losses. At a pressure of 310 mm of mercury variety *Ohanes* stored for 45 days and variety *Napoleon* stored for 35 days suffer weight losses of 1.19% and 0.63% only. Stored at normal atmospheric pressure, losses were 3.03 and 2.73%, respectively.

A new polythene device emitting sulphur dioxide appears to be promising. Bags contain a mixture of 5 g of sodium metabisulphite, 5 g of tetrapotassium-alumino-ferrite and 5 g of water. Their application enables to store the grapes as long as 2–3 months without deterioration. The quantity of sulphur dioxide absorbed by the grapes is negligible.

L. Pap

Freezing, frozen storage, freeze-drying

Meeting of Commissions C1 and C2 of the International Institute of Refrigeration, Karlsruhe, 6–8 September 1977.

Experts from 25 countries participated in the meetings and 53 papers were presented on the following topics: Basic phenomena of freezing biological materials (2 reports); Physico-chemical phenomena of the freezing process (4 reports); Effects of the

freezing process and frozen storage on animal tissue and blood (15 reports); Effects of the freezing process and frozen storage on vegetable materials and prepared foods (8 reports); Mathematical treatment of the freezing and thawing processes (10 reports); Industrial freezing of foods and distribution of quick-frozen foods (5 reports); Freeze-drying (9 reports).

Papers of the meeting concerned the most recent advances in the research on the freezing process, frozen storage and freeze-drying of foods and biological substances.

The following results merit emphasis:

Prior to the onset of rigor mortis, meat shows significantly higher water-retaining capacity than in the period after the onset of rigidity. Products made of meats prior to rigor are more tender when cooked, and loose less water during processing. This can be explained by a high concentration of ATP. High capacity of water retention can be maintained by quick freezing to prevent ATP from damage. Concentration of ATP in meats stored under −20 °C remains practically unchanged.

Freezing of meat reduces its mechanical firmness measured along the fibres. The rate of the reduction is dependent on the speed of congelation and final temperature. Speed of freezing decreases firmness of the muscular tissue more intensely than final temperature.

Fresh pork may be stored longer at lower temperatures (60 days at −5 °C, 270 days at −60 °C). Unsmoked, cured bacon has a lower shelf-life at lower temperatures: at −40 °C it can be stored for 60 days, while at −5 °C as long as 220 days. Keeping time of cured pork does not change substantially between −12 °C and −30 °C and even between −5 °C and −60 °C differences in shelf life are insignificant.

Keeping time of smoked pork and bacon depends on the temperature. The shortest shelf-life was found at −40 °C.

Quick-frozen, polythene-packed chicken retains its original tissue structure better at −30 °C and −50 °C than at −18 °C. Structural changes at these temperatures may be explained by a transformation of cell membranes and myofibrillar structure.

Differences in the quality of meat stored at −30 °C and −50 °C were negligible.

Due to the high amount of water in the protoplasm of their cells, vegetables can be stored at normal temperature for short periods only. Tomatoes, cabbage, gherkins, leeks, onions, carrot, mushrooms and parsley do not need scalding prior to freezing, since heat treatment does not improve their quality. The metabolism of Brussels sprouts, spinach, broccoli, celery, peas and green beans is very intense, therefore scalding is indispensable to the inactivation of enzymes and to the elimination of undesirable flavour components.

Frozen green beans can be stored at −18 °C for nearly one year without any significant decrease in quality. However, the vitamin C content is lowered pronouncedly during storage. The keeping time is 94 days at −12 °C, and 30 days at −6 °C.

At low temperatures microorganisms are more susceptible to ultra-violet irradiation. Susceptibility increases as the temperature decreases and reaches a maximum at about −80 °C. At this temperature, susceptibility of microorganisms is 5–7 times higher than that found at 22 °C. Mammalian cells increase their resistance against X-rays significantly, when irradiated at temperatures of −79 °C and −196 °C.

With Chinese hamster cells, a survival rate of 0.1% needs as low as a dose of 1.5 krad at 22 °C, while 4.5 krad are needed at −196 °C.

Radappertization is suitable mainly to preserve meat. Uncured meat should be treated with 40 kGy at −30 °C, while cured meat needs 30 kGy. A dose of 6 kGy is proposed to destroy *Salmonellae*.

Radurization is applicable mainly to preserve strawberries, chicken and fish. The recommended doses to treat strawberries and chicken are 2.5 and 1.5–3 kGy, respectively.

In the cold chain, temperature of frozen products fluctuates inevitably, so that the initial temperature of −18 °C or −15 °C can not be maintained to the end of the chain. The difficulty may be solved, when the temperature of storage is lowered to −25 °C or −30 °C. The spontaneous thawing (in air of 18–20 °C) may be used mainly to defrost frozen fruits. The use of tap water (at 10–15 °C) is recommended for the thawing of packaged meat and poultry. Retarded thawing in rooms adjusted at 4–6 °C is suitable to defrost bigger blocks of meats and vegetables.

Warm water (80 °C) is suitable for packaged meals, while foods of small volume may be thawed successfully by microwaves.

L. Pap

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COMPILATION OF BIOASSAY DATA ON THE WHOLESOMENESS OF IRRADIATED FOOD ITEMS

J. BARNA

(Received May 16, 1978; revision received March 5, 1979; accepted March 8, 1979)

A review of 1223 studies on the wholesomeness of some 278 different irradiated foods and feeds concerning the period from 1925 to date is presented. The compilation lists the results of the investigated parameters according to food items.

The survey of the summarized data leads to the conclusion that neither stimulative nor adverse effects of the consumption of irradiated food are consistent, unambiguous and reproducible. Neither can specific effects be related to a given food, group or level of radiation dose.

Large-scale industrial application of food irradiation depends on various factors. Of these, the main prerequisite is the safety for consumption, in addition to technological and economic advantages.

Testing the wholesomeness of irradiated food has a long history. It started by animal feeding studies carried out as early as 1925–1927 (LUDWIG & HOPF, 1925; NARAT, 1927). Up to now numerous data have accumulated internationally in this field. Since the investigation of the wholesomeness of irradiated food requires many specialized scientists and increasingly complicated complex testing systems which are time- and money-consuming processes, there is a great advantage in compiling all the data available for the hygienic evaluation of food irradiation.

To this end, in 1976 parameters investigated in various animal feeding experiments and other tests were summarized according to the neutral, deleterious/pathogenic and beneficial/stimulative effects, resp., as related to normal, physiological conditions or to control-fed untreated, or conventionally (heat *etc.*) treated food. The review (BARNA, 1976) has been distributed by the INTERNATIONAL FOOD IRRADIATION PROJECT (Karlsruhe, GFR) at request.

However, from the practical point of view, it is of interest to gather the published data according to individual food items listed in alphabetical order.

In the present study parameters involved into the wholesomeness tests are therefore grouped on the basis of their results according to food items.

From the enlisted parameters, the neutral effects are printed in roman letter type, presumably adverse effects are indicated in *italics* and beneficial influences are marked by **bold-faced** letter type in the text. Numbers after

the parameters show the reference No. in the bibliography in which the results are quoted.

It should be noted that in many cases the same parameter could be found as unchanged, adversely and stimulatively affected at the same time in the same study (*e. g.* 100, 374a, 510, 658, 793a). The reason for this is explained on pp. 263–266.

Abbreviations used in this survey

AChE	acetylcholinesterase
A/G	albumin/globulin
AP	alkaline phosphatase
ATP	adenosine triphosphate
BChE	butyrilcholinesterase
BFR	blood flow rate
BSP	bromsulphtalein
BUN	blood urea-N
ChE	cholinesterase
DL	dominant lethality
DLT	dominant lethal test
DNA	desoxyribonucleic acid
EDTA	ethylenediamine tetraacetic acid
EEG	electroencephalogram
ECG	electrocardiogram
FFA	free fatty acid
GFR	glomerular filtration rate
GOT	glutamic-oxalacetic transaminase
GPT	glutamic-pyruvate transaminase
HMA	host mediated assay
I value	iodine value
LDH	lactic acid dehydrogenase
MAO	monoaminoxidase
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MNT	micronucleus test
NPN	non protein nitrogen
OCT	ornithin-carbamyl transferase
pChe	pseudocholinesterase
PSP	phenolsulfophthalein
RES	reticuloendothelial system
retic.	reticulum
RNA	ribonucleic acid
RBC	red blood cell count
SAP	serum alkaline phosphatase
SBChE	serum butyrilcholinesterase
SDH	sorbite dehydrogenase
Se	serum
SGOT	serum glutamic oxalacetic transaminase
SGPT	serum glutamic-pyruvate transaminase
WBC	white blood cell count

1. List of irradiated foods and feeds, indicating the parameters and results of wholesomeness testing

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tested also as a component of irradiated DIET complete in 430, 688; DIET test in 693; DIET for humans in 120a

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tested also as a component of irradiated DIET complete in 221, 430, 688; DIET test in 693; DIET for humans in 120a

CORIANDER see SPICE MIXTURE

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see also PROTEIN (plant); STARCH

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tested also as a component of irradiated DIET complete in 510, 511, 599; DIET test in 247

COW PEAS see NIEBE

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(for lab. animal, lab.-chow, commercial, complex, composite, mixed, standard, stock, whole feed)

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increased backmutation frequency 374a

DIET (synthetic, semi-synthetic, purified)

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delayed maximization of hatchability 175
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increased body weight 198
reduction of weight or weight gain 204
favorable effect 571

DIET for humans (MEAL kitchen ready *etc.*, for cosmonauts, volunteer consumers)

unchanged food consumption 512
acceptance 46, 389, 553
weight gain 512
reproductive performance 512
lactation performance 512
WBC 512
haemoglobin content 512
serum A/G quotient 512
clinical chemistry 17, 120a, 430, 433, 691
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life span 512
gross pathology 512
histopathology 512
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reduced growth 512
less susceptibility to infection 512
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EGG (powder, dried, whole)

unchanged protein value 394
protein utilization 308
food consumption 308, 679
growth 308, 330, 590, 682
body weight 680
weight of offspring 590
reproductive performance 330, 590, 679
litter number at birth 590
haematological status 679
state of health 588, 589, 680
incidence of intercurrent diseases 590
life span 590
mortality of adults 590
infant mortality 330
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more frequent cannibalism 308
increased postnatal mortality 308
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tested also as a component of irradiated DIET complete in 870

EUROPEAN PLAICE FISH *(Pleuronectes platessa)*

unchanged food efficiency 221a
body weight 221a
organ weight 221a
mother weight 221a
weight of offspring 221a
reproductive performance 221a
viability 221a
litter size 221a
sex ratio 221a
lactation index 221a
RBC 221a
WBC 221a
differential WBC 221a
haemoglobin content 221a
MCV 221a
MCH 221a
MCHC 221a

unchanged haematocrit value 221a
thrombotest 221a
blood chemistry 221a
blood sugar level 221a
serum total protein content 221a
albumin 221a
BUN 221a
bilirubin level 221a
electrolytes 221a
SGOT 221a
SGPT 221a
SAP 221a
LDH 221a
leucine-amino-peptidase 221a
urine analysis 221a
LDH in urine 221a
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leucine-amino-peptidase in urine 221a
kidney concentrating ability 221a
mortality of offspring 221a
incidence of malformation 221a
gross pathology 221a
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less quick growth of females on irradiated diet 221a
relative reduction in liver weight 221a

FAT

unchanged food consumption 84
growth 555
body weight 84
haematological status 84
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peripheral chronaxy time 84
EEG examination 84
gross pathology 84
incidence of tumour 555

reduced biological value 941
reduced digestibility 582d
reduced reproductive capacity 408
disturbance in breeding performance 408
reduced sexual function in females 408
influenced motility of gastrointestinal tract 584
extended chronaxy time 84
increased mortality of progeny 408

tested also as a component of irradiated DIET complete in 236a, 430; DIET test in 937, 938; DIET for farm animals in 210

FAT (animal)

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see also BACON

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reduced fertility 518
reduced survival of offspring 518
vitamin A deficiency 749a
reduction of life span 518
encephalomalacia 749a, 755

b u t t e r f a t
unchanged growth 211
non toxic or harmful 425, 440

reduced growth 440
reproductive disturbance 211
increased mortality of offspring 211

f i s h f a t
unchanged nutritional effect 582a
non toxic or harmful 582a

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unchanged nutritive value 364
metabolizable energy 364
body weight 934
incidence of tumour 415
non toxic or harmful 93

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disturbed fat absorption 607
disturbed digestion 803
increased mortality 45
more frequent tumour incidence 45
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decreased hydrolysation activity of lipase in tissues 584
low lipid peroxidation rate 934

tested also as a component of irradiated DIET complete in 870; DIET synthetic in 370, 934

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reduced growth 749a, 755
vitamin A deficiency 749a
encephalomalacia 749a, 755

u d d e r f a t
unchanged cytochromoxidase activity in tissues 938
lack of haemorrhagic syndrome 938

further reference 558

FAT (plant)

increased growth 749a

coconut fat

EEG examination 410, 410a

tested also as a component of irradiated DIET synthetic in 345

peanut butter
unchanged food efficiency 693
growth 693
gross pathology 693
non toxic or harmful 99, 693

FEED see DIET

FISH (canned, cooked, culinary, fishery products, preserves, pasta)

unchanged food efficiency 850
protein utilization 412, 849
food consumption 93dd, 399c, 412, 850, 927
water intake 412
growth 93dd, 113a, 495a, 870
body weight 412, 927
weight gain 412
organ weights 93dd, 850
weight of offspring 850
mother weight 399c, 850
foetal weight 399c
reproductive performance 495a
preimplantation loss 399a
early embryonic death 399a
fertility 850
number of corpora lutea 399c, 850
number of implantation sites 399a, 399c, 850
litter size 850
viability of offspring 850
viable foetus 399c, 850
lactation performance 850
haematological status 93dd, 113a, 412, 495a
RBC 850, 927
WBC 850, 927
differential WBC 850, 927
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platelets 927
haemoglobin content 850, 927
MCV 927
MCH 927
MCHC 927
haematocrit value 850, 927
sedimentation rate 927
prothrombin time 850, 927
blood chemistry 113a
blood sugar level 93dd, 927
fasted blood glucose concentration 850
unchanged serum total protein content 93dd, 850, 927
albumin 850
A/G quotient 850, 927
BUN 850, 927
uric acid level 927
lipid content 927
cholesterin level 927
bilirubin level 927
electrolytes 850, 927
SGOT 412, 850, 927
SGPT 412, 850, 927
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pulse rate 927
body temperature 927
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life span 495a
mortality of adults 927
early and late implant resorption 850
stillbirth ratio 850
mortality of progeny 850
gross pathology 412
histopathology 412, 850
incidence of tumour 415, 495a, 870
mutation rate 399a
non toxic or harmful 93bb, 517
non teratogen 89, 399c, 412, 850
non cytotoxic 577a
non mutagen by DLT 399a, 850
non mutagen on animals 89

reduced biological value 836
reduced nutritive value of lipid 836
reduced protein utilization 387, 840
reduced growth rate 840
reduced weight of testicle 840
increased weight of spleen 850
disturbance in breeding performance 840
reduced activity of spermatozoids 840
extended oestrus cycle 840
more frequent cannibalism 850
increased SGOT activity 850
reduced SGOT activity 850
increased SGPT activity 850
reduced ascorbic acid content of adrenal 836
more frequent intercurrent diseases 840
higher blood sugar level at starving 850

increased mortality of progeny 840
increased excitability 84
inhibited growth of microorganisms 794

more favorable acceptance 500

further references 60, 61, 78, 79, 386, 398, 538a

see also EUROPEAN PLAICE FISH; FLOUNDER; HADDOCK; CODFISH; HAKE; HERRING; INDIAN MACKEREL FISH; OCEAN FISH; REDFISH; SAITHE; SALMON; SARDINE; TUNAFISH; TROUT; TINAPA; WHITE FISH; FAT animal

FISH EXTRACT

non mutagen 99a

FISH MEAL

unchanged nutritive value 303b, 394, 592a
protein value 394
protein digestibility 303b
organ weights 592a
fertility 592a
haematological status 592a
clinical chemistry 592a
histopathology 592a

tested also as a component of irradiated DIET complete in 3b, 100, 102, 103, 105, 109, 240, 250, 251, 253, 374a, 418, 430, 502, 502a, 510, 511, 599, 800; DIET test in 130, 133, 247, 937, 938; DIET for farm animals in 198, 212, 419

FLOUNDER (yellow tailed) FISH *(Limanda ferruginea)*

unchanged biological value 99, 713
protein quality 99, 712, 715
food efficiency 644a
protein utilization 713, 715
food consumption 93j, 644a
body weight 93j, 697a
weight gain 644a
reproduction 697a
litter weight 697a
weight of offspring 697a
body weight of mothers 697a
number of offspring 697a
fertility 697a
viability indice 697a
sex ratio 697a
stillbirths 697a
haematological status 697a
RBC 697a
WBC 697a
differential WBC 93j, 697a
haemoglobin content 697a
haematocrit value 697a
unchanged prothrombin time 697a
MCV 697a
MCVH 697a
blood chemistry 93j
serum total protein content 697a
A/G quotient 697a
BUN 697a
SGOT 697a
SAP 697a
electrolytes 697a
clinical chemistry 697a
urine analysis 697a
renal function 93j
kidney function test (PSP) 697a
liver function 93j
BSP 697a
appearance 644a
behaviour 644a, 697a
mortality of adults 93j, 697a
gross pathology 697a
histopathology 697a
non toxic or harmful 93r
non teratogen 697a

reduced protein utilization 715
elevated SAP in female 697a
more pronounced enlargement of the salivary gland 697a

increased food consumption 697a

FLOUR

unchanged food efficiency 693, 858, 885
protein utilization 858
food consumption 328, 331, 372, 721, 756, 881
acceptance 693
growth 136, 160a, 181, 331, 372, 693, 756, 881, 884, 885, 887
body weight 328, 858
weight gain 709a, 721, 724
organ weights 328, 331, 721, 724
testicle weight in progeny 787
weight of pups per litter at weaning 787, 881
weight of young animals 331
reproductive performance 136, 160a, 328, 693, 721, 724, 884, 885, 886, 887
reproductive capacity 328
lactation performance 693
fertility 328, 331, 787, 881
total number of young born 787, 881
number of young at parturition 331, 787, 881
sex ratio 331
haematological status 693, 709a, 721, 724, 787, 881, 885
RBC 721
WBC 721
differential WBC 721
haemoglobin content 721
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survival to weaning 787
mortality of progeny 331, 881
gross pathology 136, 372, 761, 787, 881
histopathology 328, 693, 724, 881, 884
incidence of tumour 136, 181
type of tumour 181
incidence of cytogenetic abnormalities 787
non toxic or harmful 99, 136, 284, 331, 693, 701, 756
non teratogen 331
non carcinogen 99, 693, 756
non cytogen 787

increased weight of spleen 328, 331
physiopathological injuries in fertility 169
reduced number of viable offspring 787
increased preimplantation loss 595
physiopathological changes in longevity 169
increased mortality of progeny 787
thyroiditis 724
more frequent tumour incidence 169, 787
increased meiotic chromosome aberration 169

improved food efficiency 721, 724
increased body weight 724
increased weight gain 721

further reference 723

tested also as a component of irradiated DIET complete in 418, 430, 502; DIET test in 693; DIET for humans in 120a

see also BREAD FLOUR

FOOD (unidentified)

unchanged nutritive value 30, 293, 379, 380, 380a, 427, 699, 701, 896
metabolizable energy 429, 431, 936a
biological value 93z
net nutritive value 700
net protein utilization 93z
protein value 494
protein quality 861d
protein efficiency 93z
digestibility 429, 431, 936a
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food efficiency 552, 689, 693
N-balance 429, 701, 936a
food consumption 552
acceptance 286, 553, 693
growth 93z, 162, 437, 441, 552, 560, 693, 699, 701, 756, 903, 932
growth rate 870
unchanged body weight 552
weight gain 113, 718
development 437
reproductive performance 93z, 162, 427, 560, 619, 693, 699, 756, 903, 932
fertility 552
lactation performance 437, 552, 693, 756
lactation index 932
haematological status 552, 560, 659, 661, 693, 932
RBC 162
WBC 162
differential WBC 162
haemoglobin content 162
prothrombin time 718
tissues enzymes 552
clinical chemistry 429, 433, 552, 691, 932
urine analysis 936a
basal-metabolism 491
total keton in the urine 936a
nitroprusside reacting substances in the urine 936a
indophenol reducing substances in the urine 936a
heart function 659
liver function 491, 659, 661
renal function 491, 552, 659, 661
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state of health 903
general health 93z
physical condition 659
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life span 162, 437, 552, 560, 693, 699, 932
mortality of adults 718
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non mutagen by HMA 70
non mutagen by DLT 70, 958
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reduced biological value 93, 344, 700
reduced protein quality 699
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retarded growth 38, 239
reduction of weight 38, 239
reduced weight gain 38, 239
reduced reproductive capacity 408
disturbance in breeding performance 242, 408
reduced fertility 36, 424
sterility 431
reduced sexual function in females 408
reduced RBC 36, 239
increased RBC 38
decreased lipid digestion 689, 700
changes in immunological reactivity 827
formation of toxic substances, radiotoxins 93, 535
increased cytochromoxidase activity in tissues 439
toxic effect 33, 928
risk in irradiated food consumption 303, 391, 816, 818
few anomalies require further research 217
more frequent incidence of cataract 239
more frequent incidence of blind individuals 431
increased mortality 36
increased mortality of progeny 38, 239, 408, 424
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rupture and dilatation of heart auricle 431
haemorrhagic diathesis 431
more frequent tumour incidence 38, 239
reduced fecundity of insects 818a
functional disorder in the thyreoid gland 892
cytotoxic effect in animal cells 610
mutagen effect on animals 535, 610

increased nutritive value 610
increased growth rate 847
increased fecundity of insect 99b

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FOOD PRODUCT OF ANIMAL ORIGIN

reduced biological value 176, 825

FOOD PRODUCT OF PLANT ORIGIN

reduced biological value 825
reduced fertility 841
reduction of life span 841
teratogen effect 841
cytotoxic effect in animal cells 841
carcinogen effect 841
mutagen effect on animals 841

FRANKFURTER

unchanged food effciency 693
growth 693
gross pathology 693
non toxic or harmful 99, 693

tested also as a component of irradiated DIET for humans in 553

FRUCTOSE

inhibited growth of plants or plant tissues 376
decreased gain in plant tissue weight 799
cytotoxic effect in animal cells 117
inhibited growth of normal animal cells 117
inhibition of microbial growth 8f
impaired respiration and oxidative phosphorylation 8f
inhibition of the labelling of protein and DNA by radioactive precursors 8f

FRUIT

non toxic or harmful 93aa

FRUIT (dried)

unchanged growth 701
reproductive performance 701
life span 701
non toxic or harmful 34, 93, 701
non carcinogen 701

further reference 383

tested also as a component of irradiated DIET test in 93, 147, 148, 148a, 149, 150, 179, 838, 937, 938, 939, 940; DIET for humans 512

see also COMPOTE (FRUIT)

GELATINE

non carcinogen 871

reduced nutritive value 741
reduced growth rate 693
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GLYCINE

increased chlorophyll mutant rate 244

GLUCOSE

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inhibited reproduction of microorganisms 451
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inhibited growth of normal animal cells 117
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increased rate of chlorophyll mutants 244
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increased sex linked lethal mutation in Drosophila 402
increased autosomal recessive lethal mutation in Drosophila 402
increased forms of phenotypic alteration in Drosophila 402
mutagen effect by HMA 392
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stimulated root formation 799
stimulated reproduction of bacteria 848
antirachitic effect 268

tested also as a component of irradiated DIET synthetic in 345

GLUTEN

corn
unchanged nutritive value 372
metabolizable energy 372
biological value 372
digestibility 372

wheat
unchanged nutritive value 372
unchanged metabolizable energy 372
biological value 367, 372, 568
protein quality 861b
digestibility 367, 371a, 568
protein digestibility 371a
protein utilization 248
non toxic or harmful 248

reduced protein value 394
reduced growth rate 787
reduced number of eggs laid 787
reduced hatchability of eggs 787

GRAIN see CEREAL

GRAPE

unchanged Saccharomyces fermentation in heart homogenization 605
Saccharomyces respiration in heart homogenization 605
non toxic or harmful 93
non mutagen by DLT 93

inhibited physiological activity of Saccharomyces 605

tested also as a component of irradiated DIET test in 137, 627

GRAPE JUICE

non cytogen in microorganisms 273
unchanged growth of microorganisms 273
physiological activity of microorganisms 273

GRASS (dried)

tested as a component of irradiated DIET complete in 179; DIET for farm animals in 198

GREEN BEAN

unchanged nutritive value 740
biological value 874
food efficiency 479, 481, 693, 704, 742
food consumption 440, 480, 481, 704, 740
acceptance 554, 693
growth 20, 144, 160a, 181, 440, 479, 480, 481, 637, 693, 701, 704, 740, 746, 750
weight gain 478a, 704, 742
organ weights 740, 746
weight of pups per litter at weaning 740
reproductive performance 20, 160a, 479, 481, 693, 701, 740, 742, 746, 750
lactation performance 480, 481, 693, 740
fertility 746

unchanged number of young at parturition 480
number of young at weaning 476, 480
haematological status 478a, 479, 481, 693, 740, 742, 746
WBC 752
differential WBC 752
haemoglobin content 752
haematocrit value 752
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transaminase 144, 637
blood alanine-alfa-ketoglutarate level 637
clinical chemistry 554
urine analysis 479, 481
bilirubin in urine 481
respiration in erythrocytes 144
glycolysis in erythrocytes 144, 637
aerobic glucose oxidation in erythrocytes 637
glutathion reductase in erythrocytes 144, 637
transketolase in erythrocytes 144, 637
state of health 181, 440
appearance 704
incidence of blind individuals 746
microphthalmy 746
life span 20, 181, 693, 701, 740, 742, 746, 750
mortality of adults 181, 480, 704, 740
gross pathology 440, 693, 704, 742
histopathology 440, 479, 481, 540, 693, 704, 740, 742, 746, 750
composition of bone marrow (smears) 752
incidence of tumour 181, 740, 750
type of tumour 181
non carcinogen 99, 693, 701
non toxic or harmful 99, 284, 543, 693, 701, 702, 704

reduced intensity of growth 746
increased spleen weight 479
fertility disorder 742

extensive weight gain 480

tested also as a component of irradiated DIET complete in 19b, 160a, 179, 430, 440, 702a, 703, 705, 950; DIET test in 693, 702; DIET for humans in 120a, 553

GREEN PEAS

unchanged food efficiency 693
growth 441, 693
gross pathology 441, 693
histopathology 441
non toxic or harmful 99, 132, 693

tested also as a component of irradiated DIET complete in 441, 706; DIET test in 130, 133, 625, 628

GRITS (mixed)

tested as a component of irradiated DIET complete in 510, 511, 599; DIET test in 247, 835

see also BUCKWHEAT; RICE

GUINEA PIG-SERUM see BLOOD SERUM

GUM ACACIA

tested as a component of irradiated DIET complete in 705

HADDOCK *(Melanogrammus deglefinus)*

unchanged biological value 99
protein quality 99, 712, 713, 715
food efficiency 31, 693, 704
protein utilization 31, 713, 715, 849
food consumption 344, 440, 704
acceptance 305, 554
growth 31, 344, 440, 441, 672a, 693, 704
weight gain 672a, 704
reproductive performance 31
clinical chemistry 554
hypotonic endogen respiration in liver 584
SAP 584
AP in liver 584
succino-dehydrogenase in liver 584
xanthine oxidase in liver 584
state of health 440
appearance 672a, 704
behaviour 672a
mortality of adults 31, 672a, 704
gross pathology 440, 441, 672a, 693, 704
histopathology 31, 440, 441, 704
incidence of tumour 674
non carcinogen 31, 672a, 676
non toxic or harmful 31, 99, 143, 184, 229, 491, 693, 702, 704, 868a

tested also as a component of irradiated DIET complete in 19b, 160a, 440, 441, 702a, 703, 705, 706, 950; DIET test in 179, 702, 937, 938, 939, 940; DIET for humans in 553

HAKE *(Merlucius merlucius)*

unchanged biological value 633, 905
digestibility 633, 905
digestible protein quality 905
net protein utilization 633, 905
food consumption 633, 905
weight gain 633, 905

HAM

unchanged nutritive value 379
digestibility 362
food efficiency 693, 704
food consumption 344, 362, 440, 704
water intake 362
acceptance 77, 304, 305, 554, 645
growth 344, 362, 440, 441, 693, 704
weight gain 704
organ weights 362
reproductive performance 362
haematological status 362
clinical chemistry 554
liver function 362
state of health 440
appearance 704
mortality of adults 362, 704
gross pathology 362, 440, 441, 693, 704
histopathology 362, 440, 441, 704
incidence of tumour 362, 379
type of tumour 362
non toxic or harmful 86, 99, 377a, 379, 693, 702, 704

retarded growth 360
reduction of weight 360
reduced weight gain 360
reduced number of pups per litter 360
reduced RBC 360
reduction of life span 360

further references 87, 92, 956

tested also as a component of irradiated DIET complete in 19b, 160a, 179, 440, 441, 702a, 705, 706, 950; DIET test in 702; DIET for humans in 553

HEMP

tested as a component of irradiated DIET complete in 606

HERRING

unchanged nutritive value 229
growth 229
organ weights 592
fertility 229
haematological status 229
incidence of tumour 229
non toxic or harmful 229

HERRING MEAL

unchanged nutritive value 593
food efficiency 593
growth 593
body weight 592
reproductive performance 592
unchanged fertility 592, 593
litter size 592
litter number at weaning 592
lactation capacity 593
haematological status 593
RBC 592
WBC 592
haemoglobin content 592
haematocrit value 592
prothrombin time 592
SGOT 592
SGPT 592
urine analysis 592
mortality 593
histopathology 592
non toxic or harmful 593

HERRING (marinated)

unchanged food consumption 801, 801a
water intake 801, 801a
weight gain 801, 801a
reproductive performance 801a, 802
blood chemistry 801, 801a
behaviour 801a
gross pathology 801, 801a
histopathology 801, 801a
non teratogen 801, 801a, 802

extended chronaxy time 801, 801a
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HISTIDINE

inhibited growth of microorganisms 811

HORSE MEAT

unchanged food consumption 327
growth 327
weight gain 322, 326
length of body 322, 326
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tested as a component of irradiated DIET complete in 94a, 94d, 100, 102, 103, 105, 109, 186, 186a, 240, 250, 251, 253, 374a, 502a, 865c, 865d, 899; DIET for farm animals in 198

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tested also as a component of irradiated DIET complete in 430; DIET test in 937, 938; DIET for humans in 512

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tested also as a component of irradiated DIET complete in 94c, 502, 902; DIET test in 937, 938

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cod liver oil

tested as a component of irradiated DIET synthetic in 370

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tested as a component of irradiated DIET synthetic in 370

oil (WISSON-OIL)

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OKRA *(Hibiscus esculentus)*

tested as a component of irradiated DIET complete in 95

ONION

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elevated SAP 93j, 93r, 399g, 399h, 554c
inhibited liver microsomal enzyme activity 112a
decreased liver aminopyrine N-demethylating and aniline-hydroxylating activity 112a

increased food consumption 112a
faster growth rate 112a
increased microsomal protein content in liver 112a

further references 399f, 554c

RED GRAM *(Cajanus cajan)*

tested as a component of irradiated DIET complete in 95, 899, 902

RED KIDNEY BEANS *(Phaseolus spp.)*

unchanged percentage of insect egg hatch 499a

RIBOSE

non mutagen by HMA 8d, 8i

inhibition of microbial growth 8f, 864b
reduced rate of respiration 864b
mutagen by in vitro microbial test 8e, 8i
impaired respiration and oxidative phosphorylation 8f
inhibition of the labelling of protein and DNA by radioactive precursors 8f

RICE (polished too)

unchanged nutritive value 93k
food efficiency 406, 642, 693
food consumption 205, 206a, 347a, 406, 642
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absorption 537
growth 193, 205, 206a, 347a, 407, 537, 693
growth rate 406, 642
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fertility 205, 206a, 407
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RBC 193, 205, 688a
WBC 193, 205, 688a
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haemoglobin content 193, 205, 688a
haematocrit value 205
clotting time 193, 688a
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early embryonal death 206a
late embryonal death 206a
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unchanged mortality of progeny 205
gross pathology 193, 206a, 693
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lack of meiotic abnormalities or chromosomal abnormalities 206a
non toxic or harmful 93k, 93s, 99, 116, 538, 688a, 693
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non carcinogen 116
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increased neonatal mortality 206a
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tested also as a component of irradiated DIET test in 147, 148, 148a, 149, 150, 625, 628

RYE

improved nutritional value 554a

tested also as a component of irradiated DIET complete in 100, 102, 103, 105, 109

RYE BREAD

unchanged acceptance by feeding 77

tested also as a component of irradiated DIET test in 130, 133

SACCHAROSE see SUCROSE; SUGAR

SAITHE FISH *(Pollachius virens* L.)

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WBC 221b
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MCV 221b
MCH 221b
MCHC 221b
thrombotest 221b
blood chemistry 221b
blood sugar level 221b
serum total protein content 221b
albumin 221b
unchanged BUN 221b
serum bilirubin level 221b
electrolytes 221b
SGOT 221b
SGPT 221b
SAP 93j, 221b
leucine amino peptidase 221b
urine analysis 221b
LDH in urine 221b
AP in urine 221b
leucine-amino-peptidase in urine 221b
gross pathology 221b

SALMON

unchanged food efficiency 31, 693
protein utilization 31, 849
growth 31, 693
growth rate 870
reproductive performance 31
mortality of adults 31
gross pathology 693
histopathology 31
non toxic or harmful 31, 99, 143, 693
non carcinogen 31

tested also as a component of irradiated DIET for humans in 553

SARDINE (dried)

unchanged sex ratio of insect 499c
survival of insect 499c
DL in insect 499c

SAUSAGE

unchanged food efficiency 693
growth 693
growth rate 870
gross pathology 693
non toxic or harmful 99, 693

worse acceptance 304, 305, 646

tested also as a component of irradiated DIET for humans in 553

further reference 893a

SEA FOOD

non toxic or harmful 31

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SHRIMP

unchanged nutritive value 379

unchanged biological value 99, 713, 874
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weight of offspring 96
reproductive performance 160a, 248, 610, 655, 687, 693, 897, 902
fertility 96, 603, 897, 898, 902
litter number at birth 897, 902
sterility 94g
total number of implantations 342a
litter size 96, 897, 902
number of young at weaning 897, 902
lactation performance 248, 610, 693, 897, 902
haematological status 96, 247d, 248, 256, 610, 655, 693, 902
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WBC 96, 248, 504
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haematocrit value 504
clotting time 248
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glutathion level 96
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transaminase activity 143, 144, 637
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blood alanine-alfa-ketoglutarate level 637
tissue function 897, 902
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glycolysis in erythrocytes 143, 144, 637
aerobic glucose oxidation in erythrocytes 637
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phospholipid contet in liver 96
peroxide formation in liver 96
total protein content in liver 96
nucleic acid (RNA, DNA) content in liver 96
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GOT in tissues 655
GPT in tissues 655
MAO in tissues 655
esterase in tissues 655
AP in tissues 655
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histopathology 96, 248, 256, 504, 610, 655, 693, 902
incidence of tumour 610, 655
lack of thyroiditis 923, 924
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non teratogen 94g, 96, 603, 898
non carcinogen 99, 693
non mutagen 957
non mutagen on microorganisms 957
non mutagen by DLT 342a
non mutagen by HMA 342a

worse acceptance 305
functional disorder of the thyroid 373

increased body weight 655
increased enzymatic digestion 603
greater pregnancy rate 342a
reduced malignity 655

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tested also as a component of irradiated DIET complete in 94a, 94b, 94c, 94d, 186, 186a, 610, 865c, 865d, 902; DIET for humans in 553

see also PROTEIN (animal)

SORGHUM

unchanged growth rate 6

SOUR CHERRY

non toxic or harmful 99, 693

SOY BEAN

unchanged food consumption 84
body weight 84
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unchanged biochemistry 84
non toxic or harmful 743
unchanged peripheral chronaxy time 84
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gross pathology 84
non mutagen in Drosophila 798

extended chronaxy time 84

improved digestibility 583

tested also as a component of irradiated DIET complete in 250, 251, 253, 260, 374a; DIET for farm animals in 210, 419

see also PROTEIN (plant)

SOYA GRITS

tested as a component of irradiated DIET complete in 3b, 240, 502a, 800

SPICE MIXTURE
(allspice, black pepper, coriander, cumin, marjoram, nutmeg, paprika)

unchanged food efficiency 108, 108b
food consumption 108, 108b, 257a
water intake 108, 108b
growth 257a
allometric growth 108
growth rate 108, 108b
body weight 108, 257a
weight gain 108
organ weights 108, 108b, 257a
unchanged RBC 108, 108b, 257a
WBC 108, 108b, 257a
differential WBC 108, 257a
haemoglobin content 108, 257a
haematocrit value 108, 257a
prothhrombin time 108, 257a
blood chemistry 257a
serum total protein content 108, 108b, 257a
A/G quotient 108, 257a
total lipid content 108b
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vitamin E 108
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detoxication function of liver 108, 257a
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protein content of liver 108b
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behaviour 108, 108b, 257a
circadian motility 108
mortality of adults 108, 108b
gross pathology 108, 108b, 257a
depot fat storage 108
histopathology 108, 257a
non toxic or harmful 93t
non mutagen by Ames test 257b

reduced growth rate 108b
slightly reduced body weight 108, 108b
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food consumption 704
growth 693, 704
weight gain 704
vitamin K supply 749
appearance 704
mortality of adults 704
gross pathology 693, 704
histopathology 704
non toxic or harmful 99, 693, 704

tested also as a component of irradiated DIET complete in 95

STARCH

unchanged metabolizable energy 584
macronutrient utilization 584
reproductive performance 889b
non toxic or harmful 93aa, 791, 889b
non teratogen 889b
non carcinogen 899b
non mutagen 889b

reduced digestibility 584, 699
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tested also as a component of irradiated DIET synthetic in 345, 370, 584, 934

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unchanged food consumption 793, 793a

unchanged growth 889a
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organ weights 793, 793a
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weight of young animals 793, 793a
reproductive performance 793, 889a
fertility 793, 793a
litter size 793a
total number of young born 793
viability of offspring 793a
sex ratio 793
lactation index 793a
incidence of life birth 793a
haematological status 793a, 889a
RBC 793, 889a
WBC 793, 889a
differential WBC 889a
haemoglobin content 793, 889a
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blood chemistry 889a
blood sugar level 793, 793a
serum total protein content 793, 793a, 889a
BUN 793, 793a, 889a
glucose level 889a
uric acid level 793, 793a
cholesterin level 793, 793a, 889a
bilirubin level 793, 793a, 889a
electrolytes 793, 793a, 889a
creatinine 793a, 889a
SGOT 793, 793a, 889a
SGPT 889a
SAP 793, 793a, 889a
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reduced RBC 793a
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increased postnatal mortality 793
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incidence of kidney cyst 793a
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altered generating time of S. cerevisiae 599a

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tested also as a component of irradiated DIET synthetic in 174, 513

potato starch
unchanged digestibility 582d

STEROL (beef, egg, pork, yeast)

unchanged incidence of tumour 415
non carcinogen 284, 430

hepatoma 345a

tested also as a component of irradiated DIET complete in 870

STRAWBERRY

unchanged metabolizable energy 490a
biological value 99
protein quality 99
food efficiency 180, 180a, 180b, 611, 693, 704
N-balance 490a
food consumption 180, 180a, 180b, 440, 611, 612, 613, 613a, 613b, 613c, 704, 947
acceptance 554
growth 180, 180a, 180b, 440, 441, 693, 704
body weight 611, 612, 613, 613a, 613b, 613c
weight gain 704, 947
length of femur 613c
organ weights 137, 180, 611, 612, 613, 613a, 613b, 613c, 947
weight of young animals 612, 613c
reproductive performance 611, 613, 613a, 613c
fertility 611, 612, 613b 613c
litter size 613a, 613c
number of young at parturition 612
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unchanged WBC 180, 910
differential WBC 180, 910
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haematocrit value 180
blood chemistry 180a, 180b
BUN 180
SGOT 611
SGPT 180
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urine analysis 180, 180a, 180b, 613a
kidney function 613a
liver function 613a
GOT in intestinal mucosa 180
GPT in intestinal mucosa 180
intestinal enzyme activities 180b
number of eggs laid 711, 613b
hatchability of eggs 611, 613b
uniform onset of laying 613b
percentage of hatch 613b
state of health 440, 611, 612, 613
appearance 704, 947
behaviour 180, 947
life span 611, 612
mortality of adults 180, 611, 704
foetal mortality 611
mortality of progeny 612, 613c
gross pathology 180, 180a, 180b, 440, 441, 611, 612, 613a, 613b, 613c, 693, 704
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increased rel. adrenal weight 613a
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reduced liver weight in female 613b
reduced liver weight in male 613c
reduced heart weight in female 613b
reduced spleen weight in female 613b
reduced weight of offspring 613c
first hatch chickens of F_1 performed poorly 613b
greater incidence of head abnormalities in semen 613b
decline in RBC 613b
decline in haemoglobin content 613b
periodical drop in egg production 613b
increased mortality 613b
increased embryo mortality 613b
increased mortality of progeny 613a, 613c
incidence of liver fatty infiltration 613b
increase in kidney concretions 613b
incidence of cystic kidney 613b
incidence of chronic nephritis 613b
more frequent tumour incidence 612
trend to higher incidence of tumours 613c
chromatid aberrations in animals 808
increased aberrant anaphase in plant cells 783

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tested also as a component of irradiated DIET test in 137; DIET for humans in 491

STRAWBERRY JUICE

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non cytogen in plant 783
non mutagen on animals 805

increased aberrant anaphase in plant cells 783
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SUCROSE (saccharose)

unchanged growth 8g, 214, 215
organ weights 214, 215
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spermiogenesis 214
biochemistry 214, 214a
phosphorylation in mitochondria in liver 214
oxidative phosphorylation of liver mitochondria 8g, 213a, 215, 216
energy requiring biosynth. process 215
succinate oxidation in liver 214
incorporation of labelled precursors into lipid and protein in liver 8g, 213a 214, 215, 216

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glycogen distribution in liver cells 214
histopathology 214, 215
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inhibited DNA synthesis 8h
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inhibited growth of seeds 404
inhibited growth of root tip per meristem 13
inhibited growth of plants tissue per cell 377, 651
decreased gain in plant tissue weight 799
increased chromosome aberration in animal cells 821
increased chromosome aberration in plant cells 404
chromatid aberrations in animals 821
chromatid aberrations in plants 404
increased meiotic chromosome aberration 339
increased aberrant anaphase in plant cells 140
cytotoxic effect in animal cells 821
antimitotic effects (retardation or inhibition of mitosis) in animal cells 821
aberrant mitosis in animal cells 339
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inhibition of the labelling of protein and DNA by radioactive precursors 8f
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inhibited growth of microorganisms 8f, 809, 810, 812
inhibited growth of pollen 401
reduced rate of respiration 864b
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increased mutation in Drosophila 338
increased dominant lethality in Drosophila 402
increased sex linked lethal mutation in Drosophila 402
increased autosomal recessive lethal mutation in Drosophila 402
increased aberrant forms of phenotypic alteration in Drosophila 402
mutagen by in vitro microbial test 8e, 8i

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tested also as a component of irradiated DIET complete in 186, 186a, 584, 800; DIET synthetic in 370, 382, 584

see also SUGAR

SUGAR

unchanged mitochondrial oxidative metabolism 214a
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non toxic or harmful 40, 214
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tested also as a component of irradiated DIET complete in 95, 236a; DIET test in 179, 937, 938, 939, 940; DIET synthetic in 345

see also SUCROSE

SUNFLOWER SEED (extracted)

tested as a component of irradiated DIET complete in 100, 102, 103, 105, 109

SWEET CHERRY

unchanged biological value 99
protein quality 99
food consumption 278, 278c, 278d
food efficiency 693
growth 693
growth rate 870
body weight 278, 278c, 278d
organ weights 278, 278c, 278d
haematological status 278, 278c, 278d
blood chemistry 278c
BUN 278
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SGOT 278
SGPT 278
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SWEET CHERRY JUICE

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unchanged survival of Drosophila larva 405
non mutagen in Drosophila 405

TINAPA

non toxic or harmful 638

TOMATO

unchanged fasting stress 93
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  organ weights 93
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  SAP 93
  urine analysis 93
  cytochromoxidase activity in liver 93
  vitamin K supply 93
  vitamin C supply 93
  state of health 93
  behaviour 93
  gross pathology 93
  histopathology 93

permeability changes in tissue hemato-membrane of different organs 93

TUNAFISH *(Thunnus thynnus)*

unchanged nutritive value 650
  food consumption 650
  food efficiency 31, 693
  protein utilization 31, 849
  acceptance 693
  growth 31, 542a, 545, 546, 650, 693
  growth rate 870
  body weight 545
  weight gain 546
  longitudinal growth of bones 545, 546
  reproductive performance 31, 545, 546, 650, 693
  lactation performance 693
  number of conceptions 545
  number of pups whelped 545
  number of pups weaned 545
  haematological status 542a, 546, 650, 693
  RBC 545
  WBC 545
  unchanged differential WBC 545
    haemoglobin content 545
    state of health 545, 650
    parasitology 545
    behaviour 650
    life span 650, 693
    mortality of adults 31
    gross pathology 545, 650, 693, 781
    histopathology 31, 545, 650, 693, 766, 781
non toxic or harmful 31, 99, 143, 229, 284, 650, 693, 701, 766
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reduced number of pups per litter 545
increased mortality of progeny 545

further reference 582b

tested also as a component of irradiated DIET complete in 221, 688; DIET for humans in 553; DIET test in 693

TURKEY

unchanged biological value 182
  protein value 823
  protein digestibility 182
  food efficiency 693, 704
  N-balance 182
  food consumption 704
  growth 693, 704
  weight gain 704
  appearance 704
  mortality of adults 704
  gross pathology 693, 704
  histopathology 704
non toxic or harmful 86, 99, 693, 704

reduced growth 704
reduced weight gain 704

UDDER FAT see FAT (animal)

VANILIA see DESSERT POWDER

VEGETABLES

unchanged food efficiency 610
  growth 610
  organ weight 610
  reproductive performance 610
  lactation performance 610
  haematological status 610
  blood chemistry 610
  vitamin K supply 748
  life span 610
  histopathology 610
  incidence of tumour 610
non toxic or harmful 93, 93aa, 99, 610
non mutagen by Ames test 419b

reduced mutagen activity of nitrosoguanidin 419d

tested also as a component of irradiated DIET complete in 94a, 94b, 94c, 94d, 186, 186a, 610, 865c, 865d, 899, 902; DIET test in 179, 838, 937, 938, 939, 940; DIET for humans in 512

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WALNUT see NUT

WATER MELON see MELON

WHEAT

unchanged nutritive value 93k, 95, 372, 668, 905
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- protein quality 861b
- digestibility 372, 633, 902
- digestible protein quality 905
- true digestibility 905
- food efficiency 94h, 95, 324, 363, 602, 610, 787, 858
- protein utilization 95, 787, 902
- net protein utilization 633, 905
- food consumption 82a, 308, 324, 348c, 633, 873b, 897, 902, 905, 909, 947
- beginning of solid food intake of pups 668
- acceptability 905
- absorption efficiency 905
- growth 82a, 93n, 94h, 95, 155a, 290, 308, 602, 610, 668, 787, 858, 897, 902, 909
- growth rate 905, 909
- body weight 324, 348c, 668, 873b, 909
- weight gain 118, 363, 633, 668, 787, 897, 902, 905, 909, 947
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- organ weights 82a, 95, 290, 291, 308, 324, 348c, 610, 668, 902, 947
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- weight of pups per litter at birth 858, 897, 902
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- litter weight 909
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- weight of young animals 95

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- survival of germ cells 729
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- preimplantation loss 186d, 610, 865b, 873a
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- development and survival of zygote 873b
- development rate of blastocytes 873a
- viable foetuses 873a
- length of gestation 668
- litter size 155a, 668
- number of young at parturition 160, 787, 858, 897, 902, 909
- litter number at birth 909
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- sex ratio 787, 858, 206d
- lactation performance 82a, 95, 308, 610, 897, 902
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increased incidence of chromosome aberration 842b
inhibition of seed germination 842b
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WHEAT BRAN

unchanged metabolizable energy 585

improved protein utilization 585
improved phosphorus utilization 585

tested also as a component of irradiated DIET complete in 3b, 800; DIET for farm animals in 212, 419

WHEAT FLOUR (biscuit)

unchanged food efficiency 602, 900
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growth 602, 900, 901
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see also WHEAT

WHEAT GERMS

tested as a component of irradiated DIET complete in 3b, 510, 511, 599; DIET test in 247

see also OIL (plant)

WHEAT GRITS

tested as a component of irradiated DIET complete in 3b; DIET for farm animals in 198

WHEAT MIDDLING

reduced growth rate 787
reduced number of eggs laid 787
reduced hatchability of eggs 787

WHEAT OFFALS (shorts)

tested as a component of irradiated DIET for farm animals in 198, 210

WHEAT PRODUCT

unchanged nutritive value 93k
gross pathology 760
histopathology
non toxic or harmful 93k

increased number of polyploid animal cells 93k

WHEY (dried)

tested as a component of irradiated DIET for farm animals in 210

WHITE FISH

unchanged food efficiency 31
protein utilization 31, 849
growth 31
unchanged reproductive performance 31
mortality of adults 31
histopathology 31
non toxic or harmful 31
non carcinogen 31

WHITE POTATO see POTATO (white)

WISSON OIL see OIL

WOOD FLOCK (pulp)

tested as a component of irradiated DIET synthetic in 370, 744, 746

XYLOSE in medium

decreased gain in plant tissue weight 799

stimulated root formation 799

YEAST (dried)

unchanged incidence of tumour 415, 870

inhibited growth of microorganisms 794

improved food efficiency 577
improved digestibility 577
improved accumulation of Ca, P 577
improved vitamin D supply 577

tested also as a component of irradiated DIET complete in 100, 102, 103, 105, 109, 240, 250, 251, 253, 502a, 934; DIET synthetic in 513, 934 DIET for farm animals in 198

see also STEROL

YEAST (dry brewers)

tested as a component of irradiated DIET complete in 3b, 374a, 510, 511, 599; DIET test in 247

YELLOW TAILED FISH see FLOUNDER FISH

ZEIN

unchanged protein efficiency 248
non toxic or harmful 248

2. Critical remarks on studies in which wholesomeness has been reported to be affected by irradiation

Analyses of the parameters grouped and reviewed on the basis of the experimental results have shown that many effects could be induced by technological and biotechnical causes independently of the radiation treatment. Unambiguous interpretation is also rendered more difficult by the problems inherent in the evaluation of summarized results.

2.1. Technological inadequacies

These can arise from a number of situations. Thus, a *radiation dose insufficient* to ensure a desired effect may result in abnormal consequences in feeding studies (882), as can a dose level which is *excessively high (e.g.* 50–100 Mrad) for practical use (476). In a number of cases variable effects of irradiation were connected with *different dose levels* applied in the treatment (8e, 34, 116, 124, 243, 248, 259b, 294, 305, 324, 328, 331, 338, 339, 345c, 347, 348a, 374, 399b, 401, 402, 417, 474, 475, 476, 527, 533, 537, 542, 612, 650, 652, 653, 654, 699, 704, 715, 721, 742, 765, 771, 773, 783, 793, 793a, 801, 808, 836, 846, 894, 910, 947, 957). *Storage time* following irradiation can also alter the results of animal feeding studies or cytotoxicity, mutagenicity tests in such a way that changes disappear during storage (34, 90, 93, 422, 542, 610, 634, 635, 636, 734b, 912a, 943, 944) or decrease gradually with it (8e, 8i, 8f, 404, 636, 911, 944). In some cases detrimental results of feeding studies may be attributed to *poor quality* or *improper preparation* of food prior to irradiation (933) or to *inadequate storage* conditions after irradiation (693). Adverse effects could be considerably reduced when irradiation was carried out in a *nitrogen atmosphere* or under *buffered conditions* at neutral pH (8h).

2.2. Experimental biotechnical inadequacies

These may also lead to adverse effects in feeding studies which could be mistaken as consequences of radiation treatment.

Many factors are connected with the **dietary regime**. One of these is the *lack of food acceptability* or a shorter or longer *adaptation period* in the food intake because of unpalatability, unusual nature of the food item for the test animal, *etc.* (108, 108b, 300). Results in feeding experiments may depend on the *level of food item* in the diet (without causing nutritional imbalance: 90, 108, 345, 652, 653, 654). *Excessive amounts* of a particular item (*e.g.* onion, milk powder, *etc.*) in the diet can induce pathological disorders (heart failure, haematologic alteration, glycosuria, *etc.*: 217, 346, 349, 431, 537, 579, 580, 679, 680). *Differences in concentration* of an item *(e.g.* sucrose solution, *etc.)* under

testing may lead to adverse or stimulative results after irradiation in experiments connected mainly with cytotoxicity, mutagenicity, *etc.* test (13, 459). Effects of irradiation may vary according to the *different formulation* of complete diets having the same chemical composition (666). *Inadequate diet* for the experimental animal made the result of the experiment doubtful (150b). *Availability of irradiated food* according to the feeding programme resulted in diverse effects in feeding studies, too (247, 510). Changes in effect could be due to the *pretreatment* (*preparation*) of irradiated food *e.g.* raw condition, heat treatment, frozen state, extraction, *etc.* (8i, 361a, 388a, 422, 511, 793, 793a, 826). *Nutritional imbalance* could be the consequence of *improper diet composition* (72, 217, 248, 316, 349, 872). Often *vitamin deficiencies* were involved in the disorders, since supplementation of vitamins reduced or eliminated the adverse symptoms (growth retardation, fertility disturbances, increased mortality, *etc.)* of deficiencies (130, 145, 159a, 175, 211, 217, 230, 242, 279, 349, 373, 408, 424, 525, 544, 545, 547, 562, 563, 573, 663, 679, 705, 708, 718, 723, 730, 732, 733, 738, 745, 787, 842a, 935, 939, 940). It is worth noting that supplementation of vitamin E did not necessarily reduce or eliminate the disorders in reproduction (408, 702, 828, 831, 835). *Supplementation* of other components of diets have also been observed to correct adverse effects in the parameters investigated. Thus addition of *antioxidants* reduced mortality and increased growth rate (755). Adverse effects in feeding studies have been eliminated by correcting *amino acid* imbalance (217, 524, 527, 613b, 723). In another case, *sucrose solution* replaced the difference between the control and tested groups (359). In some studies experimental animals could be influenced (259b) adversely (93cc, 221b, 348b, 580, 613, 613a, 631, 644a, 655, 786, 872, 915, 921a) by the *untreated foodstuff per se.* Consumption of negative (standard) and positive (containing untreated food component) *control diets* could cause differences between groups which might *mask the effect* of irradiation (47, 108, 160, 316, 346, 504, 537, 539, 611, 693, 743, 850, 918). There are experiments in which the untreated food (clam, coffee, cranberry, fish, green bean, jam, mushroom, papaya, potato, spice mixture, starch, strawberry, *etc.)* had a *non-toxic, organoleptic effect* on food intake and consequently, on body weight, relative organ weights, *etc.* This can, therefore, not be ascribed to the effect of irradiation (93cc, 93dd, 108, 114a, 129, 221a, 221b, 345c, 399e, 399f, 399h, 554b, 554c, 640, 641, 654a, 793a, 850, 872a, 873b, 917).

Among the biotechnical factors, adverse effects ascribed to ingestion of irradiated food may be derived from the **test organisms** used. Thus pathological effects observed during feeding test with irradiated food occurred *spontaneously* in animals fed on similar but non irradiated diet (149, 277, 311, 348b, 539, 579, 613a, 892, 923, 924). *Species-specific effects* can develop from diets leading to non-physiological conditions and subsequently to pathological signs *(e.g.* beef in the diet – low incidence of coprophagy – vitamin K defi-

ciency – haemorrhagic syndrome only in rats; 369, 431, 472, 527, 528, 531, 532, 533, 569, 570, 718, 743). Microphthalmy, congenital blindness, special mortality rate, *etc.* proved to be an *inherent characteristic of specific strains* in some experiments (217, 325, 349, 373, 431, 521, 522, 741, 743).

Adverse effects were established in trials in which the *numbers of animals* used had been *insufficient* (349, 877).

Results of feeding irradiated food could be altered by differences in **animal housing**. This was the case in an experiment using *bioisolation* (512).

2.3. Uncertainties of evaluation

Analysis of the summarized experimental results has frequently shown the evaluation to be open to dispute.

In some cases adverse or stimulative effects or changes in earlier experiments were *neither confirmed* when repeated (244 and 435; 339g, h, 697a and 221b; 411, 412 and 410a; 422 and 489; 542 and 573; 547 and 660; 580 and 573, 693; 555 and 660; 643 and 187, 817; 724, 923 and 760, 770, 892, 924; 740 and 660; 818a and 150b; 867 and 187, 189, 728); *nor observable* in similar subsequent experiments (93k, 345a, 348b, 349, 357, 358, 386, 422, 497, 693, 715, 730, 746, 917).

In a number of cases statistically significant effects *appeared randomly* and changes in the data of many parameters were *not consequent* and *consistent* with respect to *species* (277, 511); *sex* (between the similar sex of control and test group or between the two sexes of test group: 93cc, 129, 160, 172, 217, 259, 294, 345c, 347, 348c, 369, 399h, 411, 412, 414, 417, 431, 441, 510, 559, 641, 652, 658, 702, 723, 733, 744, 762, 764, 765, 768, 773, 783a, 839, 840, 877, 883, 884, 910, 938, 947, 957); *age* (233); *time* (118, 163, 191, 223); *successive generations* (100, 102, 103, 105, 110, 130, 160, 165, 172, 205, 206, 230, 294, 345, 346, 348b, 386, 387a, 414, 512, 513, 559, 613b, 641, 650, 653, 654, 655, 656, 705, 742, 753, 793, 819, 820, 831, 835, 839, 840, 850, 938); *pH* (805); chemicals used in *preparation* (*i.e.* extraction) of *samples* of irradiated food (374a). Similarily, during an experiment, repeated determination of a parameter (enzyme activity, blood cell count, food intake, growth, *etc.*) on the same animals in a group at various times showed different results, so the effect appeared only *temporarily* (163, 221b, 347, 386, 474, 497, 631, 653, 654, 719, 722, 730, 732, 737, 746, 793, 833, 834, 850, 911). Often in an article the author reported *different* (both adversely and favourably affected or influenced and unaltered) *results* for the *same parameter* tested on the *same food* simultaneously. These are found in many pages of the above list. These could be explained by the reasons listed above or by the surveying nature of some articles (3, 259b, 338, 377, 459, 542, 610, 715).

Observation of changes in growth, organ functions, carcinogenicity, *etc.* may be considered in many cases as *tendencies only* since in biostatistical

analyses they were not proven to be significant (42, 108, 116, 170, 204, 206, 207, 217, 221b, 324, 328, 331, 345, 357, 385, 440, 545, 613c, 640, 641, 656, 721, 787, 793a, 839, 873c, 877, 947). In some cases the authors themselves did *not* attach *biological* or *toxicological significance* even to statisticaly significant changes (135, 175, 221a, 277, 399h, 613b, 641, 652, 724, 850). They demanded *further* investigations to *confirm* adverse findings registered in their feeding studies (217, 316, 349). According to the authors some differences in the results of investigated parameters between animals on irradiated foods could be attributed to a *coincidence of undetermined local factors* (156, 221b). In the evaluation of some of the observed changes it is a very difficult task to decide on *whether* they have a *detrimental or beneficial* effect on animals (*e.g.* altered activity of some tissue enzymes, chronaxy time, intestinal flora, *etc.:* 38, 100, 163, 346, 382, 399h, 411, 430, 476, 657, 658, 693, 850, 939, 940). In connection with this *e.g.* less depot fat (and, thus, reduced body weight) due to test diet related to that of animals kept on *ad libitum* control diet is not unambiguously adverse (108, 108b). Results could not be clearly interpreted when, in spite of the statistical significance, differences between control and test groups remained *between the limits of physiological values* (631, 793a) or when morphological alterations, influenced organ weights, *etc.* were *unassociated with* any *histological, pathological* or *clinical findings* (93k, 221a, 221b, 346, 348b, 479). As to the extrapolation of experimental data, deleterious effects *in vitro* need not be parallelled *in vivo* by toxicity in the whole animal (202, 216).

Generally, it may be said that changes in the composition of irradiated food, in particular with respect to their vitamin content, or in the effects following consumption, were *no greater than* those caused by *traditional* methods of preservation such as heat treatment (19, 134, 241, 332, 333, 334, 419, 625, 665, 666, 667, 843, 844, 888). So far the consequences of traditional preservation methods have not been investigated in detail: the effects now ascribed to an irradiation treatment may not be specific to this method.

The evaluation of the summarized published bioassay data on the wholesomeness of irradiated foods leads to the conclusion that, at present, neither beneficial nor adverse effects of irradiated food consumption are consistent, unambiguous and reproducible. Neither of them can be traced back to a given food or group of foods or level of radiation dose.

*

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EFFECT OF OLIVE OIL HYDROCARBONS AND TRITERPENE ALCOHOLS ON THE STABILITY OF HEATED COTTON SEED OIL

D. Boskou and H. Katsikas

(Received January 22, 1979; accepted March 4, 1979)

Triterpene alcohols and hydrocarbons isolated from virgin olive oil were added to cotton seed oil in small concentrations. The oil was subjected to heating at 180 °C for several hours and oxidative polymerization was followed by the changes in iodine values, refractive indices, palmitic/linoleic acid ratios and viscosity. The triterpene alcohols of olive oil increased the stability of the heated oil. The hydrocarbon fraction had a similar but less pronounced effect.

The addition of olive oil unsaponifiables to sunflower oil protects it from oxidative polymerization during heating at cooking temperatures (Sims *et al.* 1972). This protective effect has been attributed to the 4α-methylsterols present in olive oil (Sims *et al.*, 1972), since naturally occurring alpha-sitosterols improve the resistance of frying oils to darkening and polymerization (Chang & Mone, 1969). Boskou and Morton (1976) reported that olive oil sterols also have some effect in retarding heat-induced changes in cotton seed oil.

The purpose of this work was to study the effect of other unsaponifiable components of olive oil such as triterpene alcohols and hydrocarbons. The additives to be tested were obtained from virgin olive oil by thin layer chromatography; each fraction was dispersed in cotton seed oil in realistic ratios and the oil was heated at 180°C±5 for 60 h.

1. Materials and methods

1.1. Materials

Samples of virgin olive oil were obtained by one of the authors from the area of Chalkidiki, Greece. Refined cotton seed oil, devoid of antioxidants or other additives, was provided by an oil plant located in Thessaloniki.

α-amyrin and squalene used as reference materials in the TLC procedures were purchased from Roth.

1.2. Isolation of triterpene alcohol and hydrocarbon fractions

The unsaponifiables of virgin olive oil were obtained by saponification and extraction of the soaps by diethyl ether (IUPAC, 1966). The solvent was

removed by evaporation on steam bath with nitrogen and a small portion of $CHCl_3$ was added. The samples were applied to a 20×20 cm plate covered with 0.5 mm layer of *Silica Gel G*. The solvent was a light petroleum, diethyl ether, acetic acid 80 : 20 : 1 mixture. The plates, after development, were sprayed with *Rhodamine* 6G and observed under UV light. Squalene and α-amyrin were used as reference materials for the hydrocarbon and alcohol fractions, respectively.

The zones containing the triterpene alcohols and hydrocarbons were cut off and extracted with ethyl ether. The ether was evaporated, each fraction was dissolved in chloroform and the whole procedure was repeated once or twice.

1.3. Heating

100 g of the oil to be tested were put into identical beakers and heated on a hot plate connected to an energy regulator. The plate was kept at 180°C±5 for 12 h during the day and switched off overnight. Samples were taken daily until heating had continued for 60 h.

1.4. Determination of physical and chemical constants

Iodine value determinations were carried out according to the method of *Wijs* (IUPAC, 1966; II D 7). Refractive indices were measured with a Jena model 32–G 110 d *Abbe* refractometer.

Viscosity values were determined at 25°C with a *Ferranti–Shirley* viscosimeter.

Methyl esters for gas liquid chromatography were prepared using AOCS method Ce 2–66 (AOCS, 1968). They were separated isothermally at 180°C with a *Hewlett–Packard* model 7620 gas chromatograph equipped with a T.C. detector. The chromatograph was fitted with a $8' \times 1/8''$ column packed with *ChromWAW*, 80–100 mesh, and coated with 20% polyethylene glycol succinate.

2. Results and discussion

Table 1 shows the changes in the physical and chemical constants of heated cotton seed oil with and without the additives. As shown in the Table, triterpene alcohols from olive oil minimize oxidative deterioration. The hydrocarbon fraction also acts as an antioxidant, but its effect is less pronounced.

Squalene makes up some 80 per cent of the hydrocarbon fraction in olive oil (GRACIAN, 1968). This hydrocarbon was also tested, after purification by preparative thin-layer chromatography, and was found moderately anti-

oxidant. This finding is in accordance with the results presented by SIMS and co-workers (1972) for heated safflower oil.

Vegetable oils are often evaluated on the basis of their unsaturation, although the latter is not the only predisposing factor in their degradation. KHATTAB and his co-investigators (1974) subjected several oils to frying temperatures (195°C) and studied their changes in relation to their unsaturation and vitamin E content. They concluded that the stability of oils and fats cannot be predicted from oxidation rates of unsaturated fatty acids in model systems. Free fatty acids and autoxidation products are important factors affecting the degradation rate.

It appears that the role of certain unsaponifiables, besides tocopherols, is not negligible and that their effect should be taken into account when the stability of heated oils is studied. A significant portion of unsaponifiables remains in the oils after refining and these compounds or their oxidation products may behave as antioxidant or pro-oxidant factors.

CHANG and MONE (1969) claimed that while tocopherols become substantially less effective or even inactive when subjected to elevated tempera-

Table 1

Oxidation of heated cotton seed oil (with and without) additives

Sample	Heating time (h at 180 ±5°C)	Viscosity (cp at 25°C)	Refractive index (n_D^{40})	Iodine value	16 : 0/18 : 2
Unheated		71	1.4645	107.5	0.41
Cotton seed oil	12	102	1.4660	101.1	0.45
Cotton seed oil	24	126	1.4672	93.2	0.57
no additive	36	213	1.4686	87.8	0.68
	48	385	1.4697	83.9	0.80
	60	771	1.4718	77.9	0.93
Cotton seed oil	12	86	1.4654	105.1	0.42
plus 0.1% olive	24	96	1.4658	103.7	0.45
oil triterpene	36	108	1.4664	99.6	0.51
alcohols	48	158	1.4675	93.5	0.62
	60	373	1.4698	85.0	0.80
Cotton seed oil	12	94	1.4656	103.9	0.43
plus 0.25% olive	24	112	1.4665	99.2	0.48
oil hydrocarbons	36	124	1.4670	97.8	0.54
	48	194	1.4681	91.3	0.65
	60	483	1.4708	81.4	0.84

tures, a small amount of alpha-sitosterols provides a fat with improved resistance to darkening and polymerization.

This effect is clearly shown in Table 2. Refined olive and cotton seed oils were heated simultaneously in this laboratory at 180°C and the viscosity

was measured at regular intervals. Cotton seed oil contained 0.02% 4α-methylsterols isolated from sunflower oil. Judging from the increase in viscosity, cotton seed oil competes with olive oil in stability, although there is a marked difference in the unsaturation between these two oils. This is entirely due to the small amount of added sterols.

Table 2

Viscosity changes in heated olive and cotton seed oils

Samples	Viscosity (cp at 25°C) heating time (h)					
	0	12	24	36	48	60
Olive oil	68	79	117	152	230	369
Cotton seed oil plus P.P2% 4α-methylsterols	71	92	115	138	218	348
Cotton seed oil (control), no additive	71	105	194	261	395	762

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EXPERIMENTS FOR THE DETERMINATION OF THE SPECIFIC RESISTANCE OF COMMINUTED AND PRESSED APPLE AGAINST ITS OWN JUICE

I. KÖRMENDY

(Received September 22, 1978; accepted March 5, 1979)

Apples of the variety *Jonathan* were comminuted and pressed in a compression – permeability cell and the specific resistance of the compressed cake at different stages of compression against the apple juice was determined. The adequate quantity of juice for the experiments has been obtained by pressing a part of the same apple variety in a batch type press and applying inactivation to the microorganisms and enzymes of the juice instantly after pressing. The slight decrease in the viscosity of this juice during storage was accounted for by applying an appropriate correction in the calculations.

The percentual juice yields belonging to different degrees of compression were approximately 20, 40, 60 and 80%. The square root of the variance of the specific resistance was found to be proportional to the specific resistance itself and thus the logarithmic transformation of resistance data could be reasonably performed. As a result of applying the appropriate fitting method the logarithm of the specific resistance varied at about the 2nd power of the juice yield.

With solid–liquid systems the specific resistance both in filtration and pressing (expression) theory (and also in other fields, like soil mechanics) can be defined as the liquid pressure difference on a somehow specified layer of the solid phase, when the liquid flows at a given volume flow rate or mass rate through the layer. Mostly, but not always, the definition includes the condition of unit viscosity.

Eqn. 1 and Eqn. 2 represent the most commonly used definition formulae (*e.g.* SHIRATO *et. al.*, 1974, KÖRMENDY, 1974a)

$$\alpha = -\frac{1}{\mu v_F} \cdot \frac{dp}{dx} \tag{1}$$

and

$$\alpha' = \frac{1}{\mu v_F} \cdot \frac{dp}{dm_s}. \tag{2}$$

In the above equations α and α' are different forms of the specific resistance (dimensions: length^{-2}, length $\times$ mass^{-1}, respectively), μ is the dynamic viscosity (dimension: mass $\times$ length^{-1} $\times$ time^{-1}), p is the pressure in the liquid phase (dimension: mass $\times$ length^{-1} $\times$ time^{-2}), x is a space coordinate (dimension: length), v_F is the size of the filtration velocity vector $\bar{v}_F$, *i.e.* volume flow rate of the liquid on unit total cross-sectional area (dimension: length $\times$ time^{-1}).

The coordinate system is specially selected, so that $\bar{v}_F$ should be directed parallel with the x coordinate axis and pointing in the direction of increasing x values. m_s is the mass of the solid phase on unit cross-sectional area, measured in opposite direction of increasing x values.

The author's expression for the specific resistance ($\mu\alpha^*$) as defined by Eqn. 3 differs from the former ones and its use is justified by special circumstances in the pressing of comminuted fruits as explained in Section 1.5.

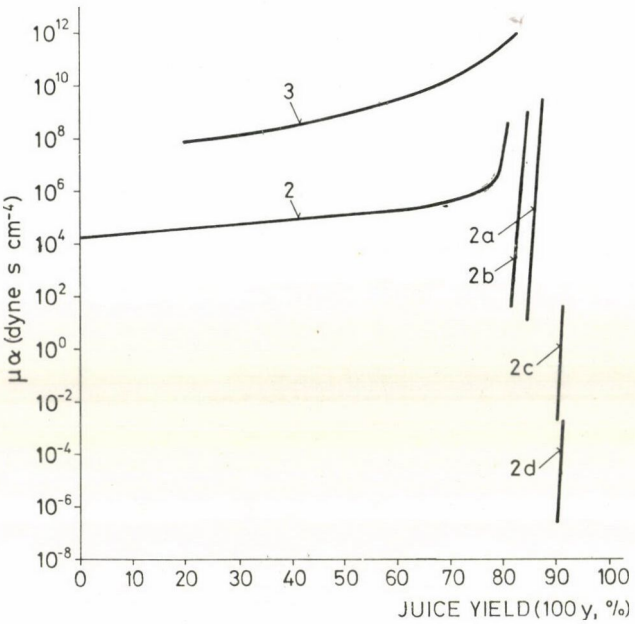

Fig. 1. Specific resistance as a function of juice yield; results obtained by different authors in experiments on comminuted apples. 1: MEPARISHVILI and ZHVANIYA (1972), *Kehura* apple; 2: KHUSID and KUPERMAN (1974), 2a: *Jonathan* apple and 2b: *Delicious* apple comminuted in the disintegrator KDP–3M, 2c: *Jonathan* apple and 2d: *Delicious* apple comminuted by means of a domestic grater; 3: the author's results, *Jonathan* apple disintegrated in a meat mincer

The specific resistance may be regarded as one of the most important physical properties in connection with the pressing operation, both in three-dimensional and one-dimensional cases (KÖRMENDY, 1974a, 1974b, 1974c). There are only a few publications on specific resistance measurements related to the pressing of fruits. MEPARISHVILI and ZHVANIYA (1972), KHUSID and KUPERMAN (1974) have measured the specific resistance of different apple varieties, at different degrees of compression. Their results are illustrated in Fig. 1 together with the author's own results for comparison. The specific resistance values as plotted in Fig. 1 have been obtained by recalculation since

the published data were given in different dimensions. The percentual juice yields, belonging to different degrees of compression of the pressed cake, have been plotted on the abscissa. The relative juice yield is understood as the ratio of the quantity of expressed juice to the quantity of the comminuted fruit before pressing and the percentual juice yield is its value multiplied by hundred. As viscosity data were partly missing, the $\mu\alpha$ values, calculated in accordance with Eqn. 1, have been plotted on the ordinate.

It is obvious from Fig. 1 that the results of different authors differ by orders of magnitude. The different comminution methods and differences in varieties do not satisfactorily explain this fact. Most probably the different experimental methods are accountable and the development of a standardized experimental method seems therefore to be highly desirable. The author's experiments and evaluation methods should be considered as one of the first attempts at improvement in this field.

Although recent publications of SCHWARZBERG and co-workers (1975, 1977) include specific resistance data on some agricultural products, results for comminuted fruits are lacking. However, he concluded that specific resistance increases very rapidly with compaction of the cake and therefore he plotted the logarithm of specific resistance *vs.* specific volume or cake height. These conclusions are in accordance with the author's results, qualitatively.

1. Materials and methods

1.1. Apparatus for the measurement of specific resistance

The general features of the apparatus are illustrated in Fig. 2. The comminuted fruit is loaded into a cylindrical chamber (1), its internal diameter being 80 mm. Both ends of the chamber are closed by metallic filter-screens and wire screens. Their specification is detailed in Table 1 and the sequence from the pressed material is A, B, C on both sides.

At the bottom end, filter screen and wire screens are supported by a perforated plate (3, 4). At the upper end of the chamber a special disc (2), serving for both pressing and juice distribution, supports the filter screen and wire screens. The disc is illustrated in Fig. 3, showing both radial and circular juice distributing channels.

The cylindrical mantle (5) is jacketed and the jacket room (6) is connected by flexible pipes (7, 8) to the water receptacle of an ultrathermostat providing constant temperature in the chamber. The receiver (9) on the letter balance (10) catches either one part of the expressed juice or the juice driven through the pressed material (cake).

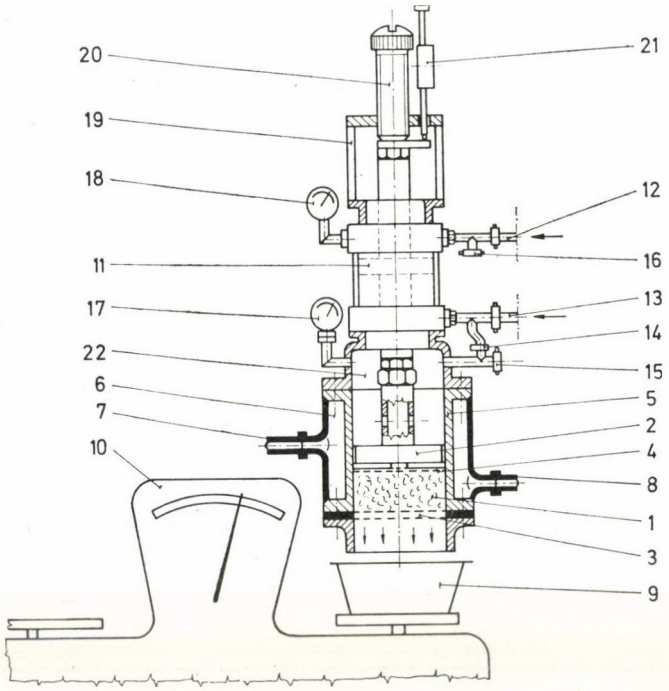

Fig. 2. Apparatus for the determination of specific resistance. 1: cylindrical chamber for the fruit pulp, 2: special disc for pressing and juice distribution, 3: filter screen, wire screens and supporting perforated plate, 4: filter screen and wire screens, 5: cylindrical mantle, 6: water jacket, 7 and 8: flexible pipes adjoining the ultra-thermostat, 9: receiver, 10: letter balance, 11: the piston of the pneumatic head, 12 and 13: pipelines for conveying pressure gas, 14: nozzle for the reduction of gas pressure, 15 and 16: cap screws, 17 and 18: manometers, 19: prop, 20: stay bolt, 21: displacement meter, 22: cover

Table 1

Screens and wire gauzes used for specific resistance measurements

Identification symbol	Specification
A	bronze screen, wire diameter 0.055 mm open space dimension of mesh: 0.17×0.17 mm
B	bronze wire screen, wire diameter: 0.25 mm open space dimension of mesh: 1×1 mm
C	bronze wire screen, wire diameter: 0.4 mm open space dimension of mesh: 1.8×1.8 mm

The apparatus is operated in two ways: Pressing (cake formation) and resistance measurement proper. The compression of the material in the chamber is performed either by pressure gas (CO_2) led above the piston (11), or by screwing the stay bolt (20) in the downward direction. The cap screw (15) is in both cases removed and so the room above the disc is connected to the

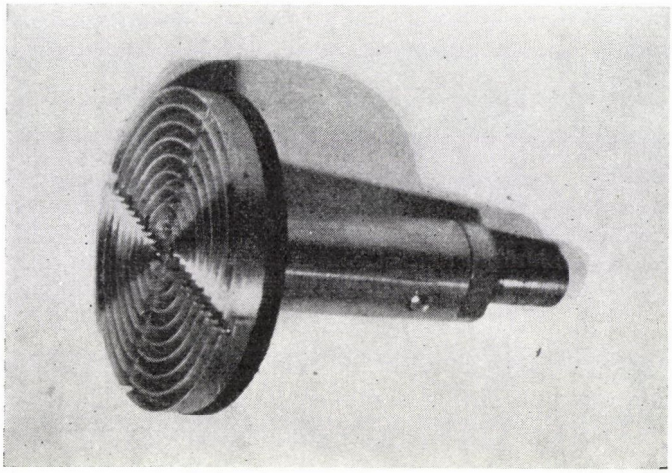

Fig. 3. Special disc for pressing and juice distribution

atmosphere. In the meantime the pipeline (13), conveying pressure gas under the piston (11) of the pneumatic head, is closed. The expressed juice flows partly into the receiver (9) and partly into the room above the disc.

In the case of compression by pressure gas the position of the piston and disc are to be fixed by the stay bolt (20) at the end of the operation to prevent the elastic backward expansion of pressed material (cake).

The displacement of the disc is measured by a displacement meter (21) of 1/100 mm scale division. Stay bolt and displacement meter are mounted on the prop (19).

The proper cake having been formed, the room above the disc will be filled with juice through the pipe stub in the cover (22). Then the stub is closed by a cap screw and pressure gas is conveyed above the level of the juice in the room through a pipeline (13) and nozzle (14). The room above the piston (11) and the adjoining pipeline (12) is pressure-free in this case.

Two *Bourdon* gauges (17, 18) serve for the measurement of pressure above the disc and above the piston.

The letter balance (10) has a scale subdivision of 0.5 mm, 50 g scale-end value and 5 kg maximum load. The temperature and relative humidity around

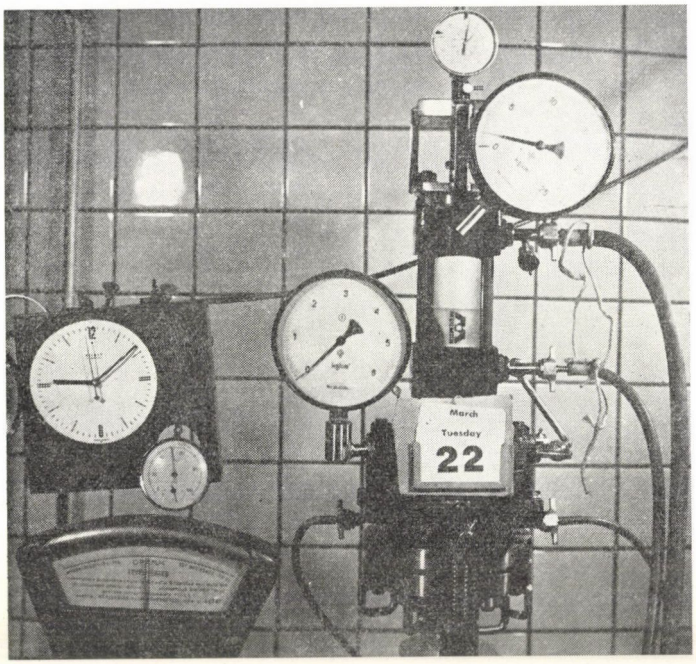

Fig. 4. A photograph of the different scales of instruments mounted on the apparatus for specific resistance mesurements

the apparatus are measured by simple room thermometer and synthetic fibre hygrometer. Time is measured by a stop watch of 1 s scale subdivision.

The scales of the different instruments are faced into the same plane and are collectively photographed in the course of the experiments as illustrated in Fig. 4.

1.2. Comminution of apple

The apple was comminuted by a meat mincer whose technical specifications are detailed in the publication of KÖRMENDY (1972), Section 1.3.

1.3. Determination of the viscosity of the expressed juice

Viscosity of the juice was measured with a modified *Ostwald* viscosimeter having a capillary of 0.95 mm internal diameter and 71 mm length. Water value at 25 °C was 48.8 s and the corresponding volume of water was 3 cm^3. The *Reynolds* number corresponding to the water value was Re = 92. Vacuum filtration of the juice prior to measurement was made in two steps,

first through a filter paper *Nagel* MN640W, then through *Nagel* MN640d paper. The density of the distilled water at 25 °C was 0.997 g cm^{-3}, viscosity 0.89 cP. Determinations were repeated three times.

1.4. Determination of the water soluble solids content, density of juice and cake

Water soluble solids content was determined at 20 °C with an *Abbe* refractometer calibrated for sucrose solutions. The density of the juice was measured in a pycnometer of 50 cm^3 volume at 25 °C.

The density of the cake was determined at 25 °C in a pycnometer by submerging the cake parts in xylol and applying deaeration, too. The density of the xylol was also determined in the same pycnometer. Detailed description of the method may be found in the publication of KÖRMENDY (1974b), Section 1.6.

1.5. Principles of the resistance measurement method

The most commonly used definitions of specific resistance have been presented in the introduction (see Eqn. 1 and Eqn. 2). However, the use of these formulae is not practical for the pressing of fruits. As for Eqn. 1, the calculation of the layer width is complicated and involves errors; as for Eqn. 2, the determination of the quantity of solids in the cake has no practical sense at all. Therefore, with regard to industrial applications and to the methods of determination, the following formula was proposed by the author:

$$\mu\alpha^* = \frac{A}{dq/dt} \cdot \frac{p_1 - p_2}{q_i/A} \tag{3}$$

and the $\mu\alpha^*$ values, referred to also as specific resistance, would always be related to the relative juice yield (y). In Eqn. 3 the area perpendicular to the direction of liquid flow is denoted by A, p_1–p_2 is the difference of liquid pressure through the layer, q_i is the initial quantity of the pressed material on area A and dq/dt is the mass rate of flow across area A. The relative juice yield stands for the characterisation of the compressed layer of the fruit (cake) and is calculated by the following expressions:

$$y = \frac{q}{q_i} = \frac{q_i - q_t}{q_i} \tag{4}$$

where q is the quantity of the juice expressed from the initial quantity of pressed material (q_i) and q_t is the related quantity of the compressed material in the layer. Figure 5 serves as further explanation and the typical form of the specific resistance as the function of relative juice yield is also illustrated, as

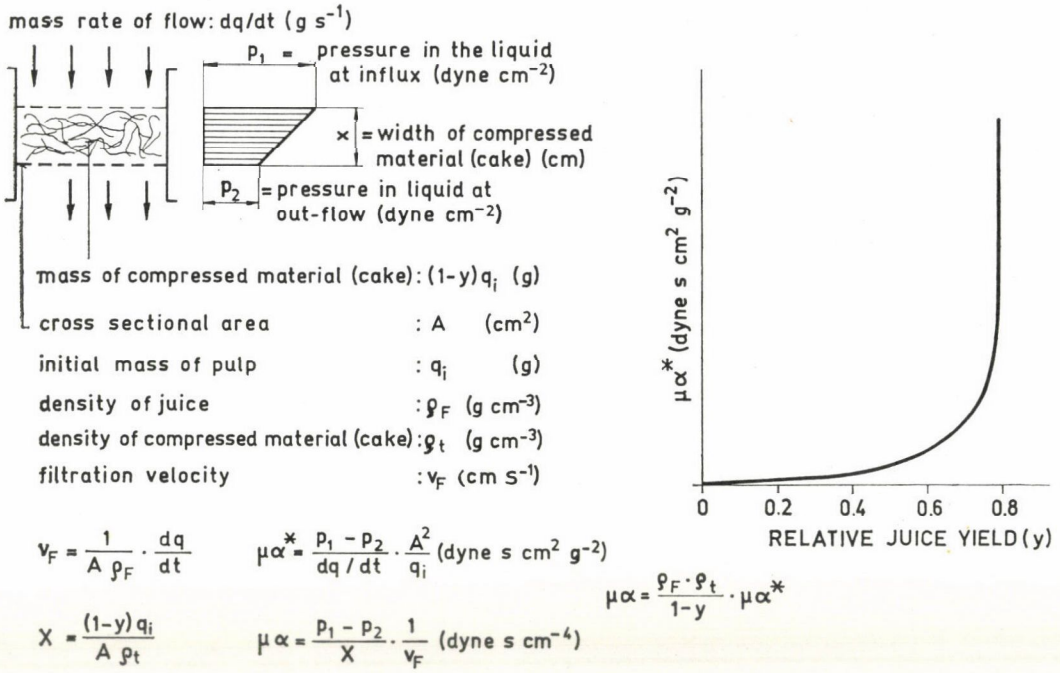

Fig. 5. Illustration of different forms of the specific resistance and its functional relationship *vs.* relative juice yield

well as the conversion formula for $\mu\alpha$ *vs.* $\mu\alpha^*$. The specific resistance expressed as $\mu\alpha^*$ is valid for the actual viscosity of the juice in the pressed material. Care should be taken in maintaining a constant temperature for the experiments and the viscosity of juice should be determined at this temperature. It seems to be more exact this time to use $\mu\alpha^*$ instead of α^* because we cannot be sure if the measured viscosity equals the actual one inside the cake as for fruit pulps.

It is important to ensure that the local values of relative juice yield throughout the layer should be constant: in this case the relative juice yield may be regarded as the equilibrium yield (KÖRMENDY, 1972).

The principle of the actual measurement procedure is illustrated in Fig. 6. The material is placed between the two filter screens (2) and slowly compressed to the previously chosen degree, then juice is filled above the cake and forced through the cake with a definite pressure. The slow compression is important to obtain uniform local compression across the cake. Having reached the appropriate compression, the cake should be fixed in its position by the stay bolt (7). The mass rate of juice flowing through the cake is measured by means of a balance and a stop watch.

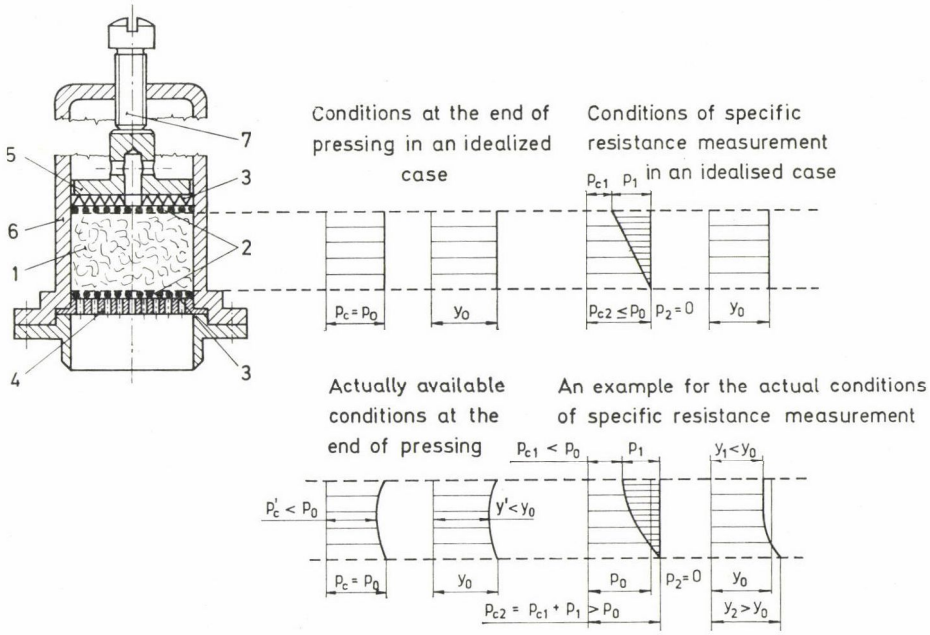

Fig. 6. Illustration of the principle of the actual measuring procedure of specific resistance. 1: the compressed material (cake), 2: filter screen, 3: wire screens, 4: perforated supporting plate, 5: pressing and juice distributing disc, 6: cylindrical mantle, 7: stay bolt; p_c, p'_c, p_{c1}, p_{c2}: different values of the deformation (cake) pressure, p_0: total (resulting) pressure, p_1 and p_2: liquid pressures, y', y_1, y_2, y_0: different (local) values of the relative juice yield

The upper part of Fig. 6 presents an idealized case when the compression is performed at a constant pressure and for a very long time and the deformation of the cake is permanent without elastic backward expansion.

At the end of cake formation the total pressure (p_0) is equal to the cake or deformation pressure (p_c) across the cake and the local equilibrium juice yield (y_0) is also constant.

When the juice has been filled above the cake and a pressure $p_1 < p_0$ is applied to force the juice through the cake, this liquid pressure will decrease in a linear way across the cake, while the cake pressure (p_c) decreases in the opposite direction from the value $p_{c2} \leq p_0$ at the outflow surface. The decrease in the local values of the cake pressure does not mean in this case a local backward expansion of the material.

The lower part of Fig. 6 presents a practically available situation. In this case the local values of relative juice yield are not uniform at the end of the compression (left side) and the deformation is partly reversibly elastic. The values of local relative juice yield will rearrange as illustrated on the right side of the lower part of Fig. 6 upon the application of the pressure p_1. The compression in the material is higher at the outflow and backward expansion

takes place at the influx. The variation of the liquid pressure across the cake will not be linear and the specific resistance as measured in such circumstances is the proper average.

The definition of the physical properties connected with Fig. 6 may be found in the publication of KÖRMENDY (1974a).

The above considerations substantiate the importance of information on the rheological properties of pressed fruit pulps. This time, disregarding correlation between equilibrium juice yield and deformation pressure in the one-dimensional case, we know only that in case of small compressions the deformation is irreversible, while at higher compressions the deformation is partly reversible, though only to a small degree. Therefore the publications of SHIRATO and co-workers (1974, 1977a, 1977b), who applied the *Hooke* and *Voigt* model in cascade connection in chemical engineering, should be taken into consideration.

1.6. Preliminary experiments for the prevention of viscosity change of the expressed apple juice during storage

The experiments lasted from April 1 to June 22, 1977. Ten kg Jonathan apple were comminuted according to Section 1.2. The apple pulp was pressed in a batch-type wine press lined with terylene filter-cloth. Samples were taken from the juice during the pressing operation and viscosity at 25 °C and water soluble solids content at 20 °C was measured.

The expressed juice was divided into two parts. One part, with 0.2% sodium benzoate added, was stored in a closed receptacle at temperatures between 0 to 3 °C without any further treatment. The other part was heated to boiling point in 28 min, and kept boiling for 7 min, then cooled to about 30–35 °C. Next, 0.1% sodium benzoate, 0.1% tannin and 0.1% phosphotungstic acid was added and stored under the same circumstances as the first part.

The viscosity at 25 °C and water soluble solids of the stored juices were measured during 82 days every 4 days on the average.

Measurement data were evaluated as follows:

It was assumed that the viscosity of the juice varies, in accordance with the theory of kinetic reactions of the first order, as a negative exponential function of storage time; the proper expression being

$$\mu = \mu_\infty + (\mu_i - \mu_\infty)\, e^{-kt} \tag{5}$$

In the above equation μ_i is the initial viscosity, μ_∞ is the viscosity after a very long (theoretically infinite) storage time, k is the velocity constant of viscosity change and t is the storage time.

This type of function was fitted to the measurement data with the least-squares method, minimizing the sum of squares of deviation of viscosity from the fitted function values. Thus, the three constants of Eqn. 5 (μ_i, μ_∞, k) were obtained. Practically the fitting has been based on the viscosimeter outflow time and the results were transformed to viscosity values.

It is worth mentioning that the calculations were executed by running a linear regression programme on the Hungarian-made calculator EMG666. This was possible by choosing a preliminary fixed value for k and running the programme by adopting the negative exponential values of kt for each t. Calculations were then repeated with step-wise increased k values as long as the minimum sum of squares has been found. Attention should be paid to the fact that the degree of freedom for calculating the mean estimated variance around the fitted line is 24–3 as there are 3 constants to be determined from 24 storage times.

1.7. Detailed description of the specific resistance measurements

1.7.1. Preparation of apple juice. Apple juice was prepared from November 8 to November 9, 1977. 40 kg *Jonathan* apple of good quality have been stored between 0 to 5 °C and 20 kg were randomly selected from this lot, quartered after the removal of stems and comminuted in accordance with Section 1.2. The comminuted apple was pressed in a manually operated wine press lined with tightly woven terylene cloth. The viscosity and water soluble solids content of the juice (2.314 cP at 25 °C and 12 refr. %) were immediately determined after pressing. The juice yield was 48.5%. Next the juice was heated to boiling point with electric heaters, then cooled to 30–33 °C, and 0.1% sodium benzoate, 0.1% tannin and 0.1% phosphotungstic acid were added. Water loss from evaporation was compensated by adding sterilized distilled water restoring the soluble solids content to a refractometer value of 12%. The juice was stored between 0 to 5 °C.

On the following day fibrous parts were separated by centrifuging in the batch type separator (type Bd 1 of the *Zuglói Gépgyár*, now *Budapesti Vegyipari Gépgyár*, at a speed of 2850 rpm, acceleration at the outermost point of the tube per gravitational constant: 2040). Centrifuging lasted for 15 min and acceleration and braking needed 2 more min. Fibrous parts were about 1% and the viscosity of the centrifuged juice was 1.942 cP at 25 °C and this value remained constant for the period of the experiments. The juice was stored again under the previously mentioned conditions and used for resistance measurements.

1.7.2. Measurement of the specific resistance. The experiments were carried out from 15 to 22 November 1977. Five kg of apple were selected randomly from the remaining 20 kg of stored apple (see Section 1.7.1.). From each

apple a segment of 1/6 part was cut, containing a corresponding part of the core, the stem being previously removed. The remaining part of the 5-kg lot was placed back in the storage room. Comminution was done according to Section 1.2. One hundred and twenty five g of the apple pulp were measured into a glass vessel and 0.1% sodium benzoate, 0.1% tannin and 0.1% phosphotungstic acid were added and carefully mixed. This sample was filled into the cylindrical chamber of the apparatus described under Section 1.1. Losses of a few tenths of a g were allowed for. Care was taken to keep the same inner height of the cylindrical chamber at each experimental run by means of the displacement meter (see Table 4). Next the chamber was closed in accordance with Section 1.1. and turned from the downward filling position into the upward measurement position. Instruments and receiver were also placed in their proper position. Now the first cake was formed by moving the disc slowly downward by about 15 mm by means of the stay bolt (see Fig. 2, number 20). The displacement meter served here also for the exact positioning of the disc. 200 cm^3 of the stored juice was heated to 25 °C and filled above the cake, then it was forced through the cake with the appropriate pressure. The mass of juice on the balance, the time, the pressure and other values as listed in Section 1.1. were measured simultaneously by photographing the instruments. The above procedure was repeated three more times, in each case the disc was moved about 5 mm downward (the 15 mm downward movement in the 1st stage was needed to expel the air from the pulp).

The last measurement having been finished, the cake was carefully removed from the chamber and its quantity and width were measured.

The ultrathermostat was regulated to keep water temperature at 25–25.2 °C.

1.7.3. Evaluation of specific resistance measurement data. The relative juice yields for the characterisation of the degree of compression of the cake were evaluated by the following formula:

$$y_j = \frac{q_i - q_{tu} + (E_u - E_j) A \varrho_F}{q_i} . \qquad (6)$$

Here y_j is the relative juice yield belonging to the cake of compression stage j, q_i is the initial quantity of the pulp, q_{tu} is the quantity of the last (4th) cake, E_u is the displacement meter value of the last cake, E_j is the same for stage j. The serial number j varies from 1 to 4. ϱ_F is the density of the juice and A is the cross-sectional area of the chamber. Pertinent values are presented in Tables 4, 5 and 6.

The specific resistance was calculated in accordance with Section 1.5. from the expression:

$$\mu\alpha^* = KA^2 \frac{t}{q_m} \cdot \frac{p_1 - p_2}{q_i} \tag{7}$$

where p_1–p_2 is the pressure drop across the cake, q_m is the quantity of juice which appeared on the balance at time t. K is the ratio of the viscosity of the previously prepared juice immediately after pressing to the actual viscosity of the juice during the experiment. Other symbols have been previously explained.

The value of K was calculated for 2.314/1.968 = 1.176 in accordance with Section 1.7.1. and Table 5.

The specific resistance from Eqn. 7 is a corrected value in view of the viscosity of the freshly pressed juice.

A more exact formula, which includes evaporation losses from the juice is the following:

$$\mu\alpha^* = KA^2 \frac{t}{q_m + v_p t} \cdot \frac{p_1 - p_2}{q_i} \tag{8}$$

where v_p is the evaporation rate. However, evaporation was neglected as the rate could be estimated for $v_p = 7.8 \cdot 10^{-3}$ g min^{-1}, which means about 0.25 g juice loss in an average measuring time of 30 min (see KÖRMENDY, 1974b).

2. Results

2.1. Results of preliminary experiments to determine the viscosity variation of apple juice during storage

Table 2 presents the viscosity and other related data obtained on the day of pressing the apple. Figure 7 and Table 3 present data on the two types of apple juice during storage and the results of the applied fitting method.

2.2. Results of the specific resistance measurements

The major measurement data, such as initial quantity of pulp and mass of the last cake, displacement meter values and pressure differences, juice quantities and pertaining time values are presented in Table 4. Table 5 contains the physical properties of the juice and data on the laboratory room. Table 6 presents specific resistance data and the pertaining percentual yields, while Fig. 8 illustrates the logarithms of resistance data *vs.* per cent juice yield together with the fitted curve (broken line) and the expected value of specific resistance as a function of the yield (full line).

Table 2

Viscosity and other properties of the apple juices obtained by pressing
(April 1, 1977)

No. of sample	Time of sampling from the start of pressing (min)	Viscosity at 25 °C (cP)	Water soluble solids content (ref. %)	Note
1	2	2.34	12.4	Samples taken during the pressing operation
2	42	2.79		
3	79	3.02		
4	116	3.09		
The average of No. 1 to 4	—	2.81	12.4	
5	ab. 140	2.9	12.6	From half of the expressed juice, 0.2% sodium benzoate added
6	ab. 200	2.88	12.6	From the other half of the expressed juice. Treatment in accordance with Section 1.6.

Table 3

Results of the fitting method applied to viscosity data of apple juices stored at 0–3 °C
(April 1–June 27, 1977)

Previous treatment	μ_∞ (cP)	μ_i (cP)	Velocity constant k (day^{-1})	The equation obtained from fitting, μ = viscosity (cP) t = time (days)	Standard deviation from fitted values s (cP)	Coefficient of variation related to μ_∞ $100s/\mu_\infty$	Water soluble solids content (ref.%)	Number of data points
See Table 2 No. 5	1.205	2.891	0.99	$\mu = 1.205 + 1.686\, e^{-0.99t}$	0.055	4.56	12.50	24
See Table 2 No. 6	2.625	2.879	0.145	$\mu = 2.625 + 0.2524\, e^{-0.145t}$	0.095	3.62	12.71	24

Table 7 presents the results of mathematical statistical calculations connected with the experiments. The specific resistance was calculated from Eqn. 7 and expressed in dyne · s · cm^2 · g^{-2}, *i.e.* pressure difference was converted into dyne · cm^{-2}, mass rate of flow into g s^{-1}; initial mass of pulp was expressed in g, area in cm^2.

Table 4

Major measurement data from experiments for the determination of specific resistance
(November 15–22, 1977)

Date of experimental runs		November 15	November 16	November 17	November 18	November 21	November 22		
Quantity of pulp + additives loaded in the chamber in grammes (q_l)		124.975	124.975	124.875	127.875	125.075	124.975		
Initial value on the displacement meter in cm (E_i)		2.0467	2.0466	2.0466	2.0472	2.0467	2.0470		
The displacement meter value at the end of cake formation in cm (E_j)									
Stage number of the cake	1	3.5655	3.5690	3.5690	3.5690	3.5691	3.5690		
	2	4.0591	4.0591	4.0591	4.0593	4.0595	4.0592		
	3	4.5585	4.5590	4.5589	4.5591	4.5589	4.5592		
	4 (E_u)	4.9840	4.9770	4.9754	4.9752	4.9760	4.9767		
Mass of the last (4th) cake in grammes (q_{tu})		26.075	27.100	27.800	26.900	25.900	26.250		
Pressure difference in kp/cm² (p_1–p_2) Mass of juice on the balance in grammes (q_m) Time interval in min (t)							Repetitions on a fixed cake were performed 3 times on November 22, applying various pressure differences		
Stage number of the cake	1	0.203	0.252	0.251	0.250	0.252	0.100	0.196	0.247
		108.10	138.00	111.30	144.90	150.60	22.40	26.10	40.50
		8.99	25.83	34.52	41.00	29.34	7.00	4.00	4.87
	2	0.500	0.500	0.502	0.504	0.508	0.301	0.400	0.500
		76.80	78.90	87.00	80.60	83.10	24.50	43.60	45.40
		22.01	34.01	30.00	39.00	21.00	10.00	14.00	12.00
	3	1.000	1.005	1.003	1.000	1.002	0.599	0.804	1.005
		17.84	17.80	30.50	27.20	41.40	10.60	14.85	19.90
		42.00	38.00	59.01	50.60	39.92	19.00	21.00	26.00
	4	2.000	2.000	2.000	2.000	2.000	1.200	1.600	2.000
		3.80	4.85	8.30	6.50	7.80	2.200	2.50	4.25
		61.01	66.00	88.00	94.00	31.00	30.00	30.00	45.00

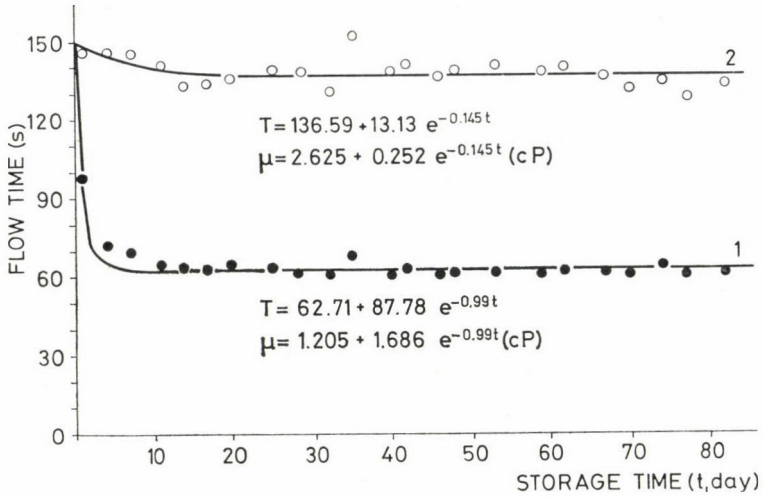

Fig. 7. Variation of viscosimeter flow time *vs.* storage time for apple juice, applying two types of treatment. 1 (●): 0.2% sodium benzoate was added to the juice immediately after pressing, 2 (○): heat treatment and the subsequent adding of 0.1% sodium benzoate, 0.1% tannin and 0.1% phosphotungstic acid to the apple juice after pressing. Storage temperature: 0 to 3 °C. Experiments were carried out from April 1 to June 22, 1977

Table 5

Properties of the apple juice and cake used in the experiments and laboratory conditions

Density of apple juice at 25 °C in g cm^{-3} (ϱ_F)	1.048
Water soluble solids content of apple juice in ref. %	12.40
Viscosity of apple juice during the experiments in cP	1.968
Viscosity of apple juice immediately after pressing in cP	2.314
Density in g cm^{-3} of the last cake at 79% yield of juice	1.0724
Cross-sectional area of cake in cm^2 (A)	50.2655
Room temperature around the apparatus in °C	16–19
Relative humidity around the apparatus in %	58–76

Table 6

Values of the specific resistance ($\mu\alpha^*$) *and pertaining percentual juice yields* (100y)

Date	No. of cake	Percentual yield	Specific resistance in dyne · s · cm^3 · g^{-2} if tabulated values are multiplied by 10^3		
November 15	1	19.35	0.2366		
	2	40.16	2.0049		
	3	61.21	33.6393		
	4	79.14	450.1995		
November 16	1	18.97	0.6595		
	2	39.63	3.0173		
	3	60.70	30.0452		
	4	78.32	380.4679		
November 17	1	18.41	1.0916		
	2	39.09	2.4241		
	3	60.17	27.2023		
	4	77.74	296.8731		
November 18	1	19.14	0.9907		
	2	39.82	3.4148		
	3	60.91	26.7862		
	4	78.46	404.9832		
November 21	1	20.04	0.6869		
	2	40.70	1.7949		
	3	61.73	13.5115		
	4	79.30	204.5314		
			Repetitions on a fixed cake		
November 22	1	19.66	0.4369	0.4200	0.4144
	2	40.33	1.5261	1.7978	1.8496
	3	61.40	15.0275	15.9278	18.3878
	4	79.00	229.1524	268.7367	296.6299

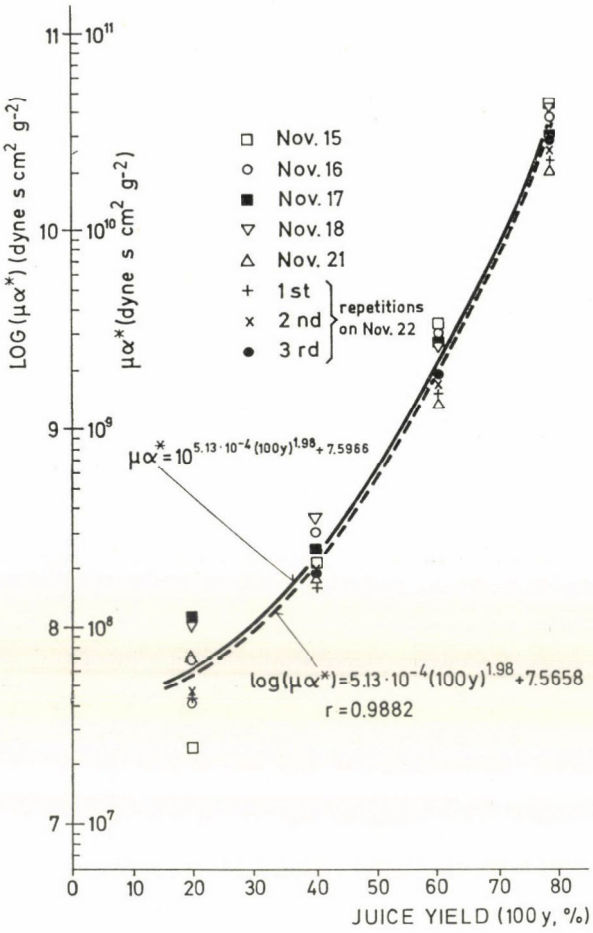

Fig. 8. Measured values of specific resistance of compressed apple pulp *vs.* per cent juice yield, the result of the fitting method used for the logarithms of specific resistance and the expected values of specific resistance as the function of per cent juice yield. The broken line illustrates the result of the fitting method and the scale on the left side of the ordinate is related to this curve. The full line illustrates expected values of specific resistance (as calculated from the fitted ones using Eqn. 11) and the scale on the right side of the ordinate is related to this curve. Experiments were carried out from November 15 to 22, 1977

Table 7

Results of the statistical evaluation of specific resistance data

Groups		Stage number of cake				Mean
		1	2	3	4	
Group means of percentual yield		19.2617	39.9550	61.0200	78.6600	49.7242
Results for $\mu\alpha^*$ in dyne · s · cm^2 · g^{-2}	Group mean (multiplied by 10^8)	0.6571	2.0080	22.5660	316.4456	85.4092
	Variance (multiplied by 10^{16})	0.0898	0.9495	59.7426	7574.6034	1908.8463
	Standard deviation (multiplied by 10^8)	0.2997	0.9744	7.7293	87.0322	—
	Coefficient of variation in %	48.6	48.5	34.3	27.5	39.7
Results for log ($\mu\alpha^*$)	Group mean	7.7431	8.3324	9.3302	10.4857	8.9729
	Variance	0.0489	0.0149	0.0236	0.0145	0.0255
	Standard deviation	0.2211	0.1221	0.1536	0.1183	0.1597
Bartlett's chi-square	Calculated from the variances of $\mu\alpha^*$ $129.87 > 6.25$			Calculated from the variances of log ($\mu\alpha^*$) $3.45 < 6.25$		
Tabulated chi-square	Degree of freedom: 3, probability level: 10%, chi-square = 6.25					

3. Conclusions

3.1. Conclusions on preliminary experiments to determine viscosity variation of apple juice during storage

It is obvious from Fig. 7 and Table 3 that there is no significant difference between initial viscosities obtained from the fitting method (μ_i), while the difference between limit viscosities (μ_∞) is highly significant (levels of significance: 5 and 1%). Thus the viscosity of the juice to which only sodium benzoate was added decreases rather fast from 2.991 cP to 1.205 cP, which is 42% of the initial value.

The viscosity of the juice which was boiled and treated with other chemicals changed from 2.879 cP to 2.625 cP, which amounts to 91% of the initial value. As a conclusion, the latter method seemed to be appropriate for preparing apple juice for specific resistance experiments.

There is also a large difference between the velocity constants (k).

However, no test of significance was conducted here as the method is currently missing and the question is of minor importance.

3.2. Conclusions regarding the determination of the specific resistance of apple pulp

It is obvious from Table 6 and Fig. 8 that the relative juice yields which belong to the same stage number (j) differ only slightly from one another. It is possible therefore to group the resistance data according to the stage number, calculate the variances of specific resistance within the groups and test them for homogeneity with *Bartlett's* method. As seen in Table 7 the hypothesis of homogeneity had to be rejected as the calculated chi-square is higher than the tabulated one.

Moreover, a simple plotting of the standard deviations of the groups against the average resistance of the groups presents a linear proportionality between the two variables. Therefore, the logarithmic transformation of specific resistance data seemed well substantiated. The *Bartlett* test in this case proved the homogeneity of variances as presented in Table 7. Thus, in Fig. 8 the logarithms of the specific resistance were plotted against per cent yields and the curve in the Figure has been obtained by fitting the relation:

$$\log (\mu\alpha^*) = a + b\,(100y)^c \tag{9}$$

to measured values by means of the method of least-squares. a, b and c are constants in Eqn. 9, while $100y$ is the percentual juice yield. The resulting relation was:

$$\log (\mu\alpha^*) = 5.13 \cdot 10^{-4}\,(100y)^{1.98} + 7.5658 \tag{10}$$

where $\mu\alpha^*$ stands in dyne $\cdot$ s $\cdot$ cm^2 $\cdot$ g^{-2}.

The mean variance of the logarithms of the specific resistance from the fitted curve was

$$s^2 [\log (\mu\alpha^*)] = 0.0288,$$

the degrees of freedom for its calculation were 32–3 = 29 as three constants were determined and altogether $4 \times 8 = 32$ pairs of data exist.

The mean variance of logarithms from the fitted curve is only slightly higher than the mean variance of logarithms of specific resistance values of the groups in Table 7 ($\bar{s}^2 = 0.0255$). Therefore the fit can be accepted.

The calculation was made with the calculator EMG666 and a programme for linear regression was applied by inserting logarithmic transformation for specific resistance values and raising the per cent yields to a previously chosen power (c). The first value of c was chosen for 1 and the programme was repeatedly run, increasing the value of c each time by 0.01, until the sum of deviations have reached minimum.

The problem of how to calculate the expected values of specific resistance as a function of percentual yield, was solved in the following way: Assuming that at a fixed value of y the values of log ($\mu\alpha^*$) from measurements are normally distributed around the fitted value calculated from Eqn. 10, the original $\mu\alpha^*$ values are distributed in a log-normal way. Applying the appropriate calculations for log-normal distributions from literature (PRÉKOPA, 1962) the expected values of specific resistance can be expressed in the following form:

$$\mu\alpha^* = e^{\frac{1}{2}[\ln 10 \cdot s(\log \mu\alpha^*)]^2} \cdot 10^{5.13 \cdot 10^{-4}(100y)^{1.98}+7.5658} = 10^{5.13 \cdot 10^{-4}(100y)^{1.98}+7.5988}. \quad (11)$$

The variance of $\mu\alpha^*$ will be:

$$s^2 (\mu\alpha^*) = [e^{(\ln 10 \cdot s(\log \mu\alpha^*))^2} - 1] (\mu\alpha^*)^2 = 0.165 (\mu\alpha^*)^2 \quad (12)$$

and the standard deviation:

$$s (\mu\alpha^*) = 0.406 \mu\alpha^* \quad (13)$$

This result is, of course, in conformity with the previous hypothesis of linear proportionality between the two variables. Eqn. 13 also shows that the coefficient of variation is independent of the specific resistance itself and has a rather high value (40.6%). This value differs only slightly from the mean of the coefficients of variation of the groups in Table 7 (39.7%).

In Fig. 8, the broken line illustrates Eqn. 10, while the full line illustrates Eqn. 11, and no considerable difference exists.

*

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MATHEMATICAL MODEL FOR THE MANUFACTURE OF FRANKFURTER TYPE SAUSAGES

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Frankfurter type sausages are manufactured in Hungary on a large scale, therefore, it is essential that this be done in a profitable way. The cost of the raw materials has to be set at a minimum while still satisfying the requirements of chemical composition as specified in the standards.

A mathematical model was developed permitting the calculation of optimum formulae as a function of the chemical composition of the raw materials. The scatter arising in the raw materials and from the manufacture has been built in the system of equations and thus these are not linear. Apart from the development of formulae, the model may be used in the modelling of theoretical problems and in profitability analyses.

In Hungary, *Frankfurter* type sausages belong to the group of "red" sausages. Beside *Wieners* the *Pariser* is also ranged in this group, the latter being of a relatively large diameter and consumed cold. The *Polony* ("krinolin") and *Knackwurst* ("szafaládé") belong to this group, too, they are of medium diameter and are consumed, just as *Wieners*, hot.

The emulsions used in these products have common characteristics, thus their chemical composition and manufacturing technology are alike, too. The meat is homogenized while adding polyphosphate, a mixture containing nitrite and salt, water, seasonings and finally some bacon. It is essential to achieve a homogeneous fine consistency, mostly by the use of a cutter and fine cutter (*e.g. Stephan*) (LŐRINCZ & LENCSEPETI, 1973).

Beside the sensory properties, the standard chemical composition is the main requirement. To ensure this is necessary from the consumer's point of view and also in the economic interest of the manufacturer. Exceeding the minimum protein requirement, beyond the level justified by the scatter, causes a superfluous cost increase.

The final composition is naturally mainly determined by the composition of the raw materials. Thus the knowledge of the initial parameters and the following up of the changes during processing permits the analysis of relations to the final composition. The model of the manufacture of the product serves this purpose. If in the model the initial and final parameters are known (by analysis and by standard specification, respectively), the dependent variables provide the optimum formula as a solution.

1. Basic relations

1.1. Development of the system of equations

The quality of the products is, thus, laid down in the related standards. In developing the model the chemical composition and sensory properties as described in the standard have to be taken into account. Consideration of the chemical characteristics seems to be the most simple. Since the quantity of the additives (salt, polyphosphates, seasonings) is constant, only the protein and water contents of the meat and bacon have to be accounted for. The standard specifications for fresh sausages are as follows (Hungarian Standard, 1976):

	in one sample	in the average of 4 samples
protein	$\geq 9.5\%$	$\geq 10.5\%$
water	$\leq 70.5\%$	$\leq 69.5\%$

For the sake of simplicity let us assume that the chemical composition of the sausage emulsion is identical with that of the ready-made product and let us not consider the scatter either. If 1 kg emulsion is composed of α kg of meat, β kg of bacon and γ kg of water, then, given the percentage moisture and protein contents of meat and bacon (W_M, P_M, W_F, P_F), the following equations are valid for the average of 4 samples:

$$\alpha P_M + \beta P_F \geq 10.5 \qquad (a)$$

$$\alpha W_M + \beta W_F + 100\gamma \leq 69.5 \qquad (b)$$

Since we reckon with 1 kg of emulsion and the emulsion contains about 2.5% of additives (salt + polyphosphates + seasonings)

$$\alpha + \beta + \gamma = 0.975 \qquad (c)$$

Thus, the specifications of the standard in relation to chemical composition were taken into account (ZUKÁL *et al.*, 1964; KÁRPÁTI & ZUKÁL, 1967; ZUKÁL, 1967; ZUKÁL & KÁRPÁTI, 1967).

The most important characteristic of the sensory quality is the consistency of the fresh sausage which depends basically on the water binding and emulsifying capacity of the meat utilized. This factor is a function of the *quantitative* and *qualitative* composition of protein. However, for the time being, the relation between the protein quality and the water binding capacity of the meat cannot be described by a mathematical equation, inspite of the fact, that the problem has been discussed widely in the literature (HAMM, 1972). This draws attention to the necessity of developing such a method for the determination of the water binding capacity of meat, which is in a close correlation with the amount of water that may be added to the meat.

Thus, in the first approximation the addition of extraneous water is formulated as a function of the protein content of meat. On the basis of practical experiences, to a lean beef containing 21.4% protein and 75% water, only 80% extraneous water may be added: with meats of lower protein content (and higher fat content) proportionately less water can be used. The water-binding capacity of the protein content of bacon is neglected for the time being, since generally the adipose tissue contains proteins of lower water-binding capacity:

$$\frac{80 + 75}{21.4} = 7.24$$

Thus:

$$\frac{\alpha W_M + 100\gamma}{\alpha P_M} \leq 7.24 \quad \text{and}$$

$$\alpha W_M + 100\gamma \leq 7.24\,\alpha P_M \qquad (d)$$

The aim is to minimize the cost of the raw material required for the emulsion. If the emulsion is prepared from meat and bacon the objective function is

$$C = \alpha C_M + \beta C_F \rightarrow \min. \qquad (e)$$

where

C_M is the price of meat,
C_F is the price of bacon.

The price of water may be neglected. Equations (a) to (d) are, in fact, conditional equations and the task belongs to the field of *linear programming*.

Since $\alpha + \beta + \gamma =$ constant, the value of γ in the equation may be substituted into equation (b) or (d) and thus these equations contain two variables and may be solved graphically.

1.2. Example of calculation

Let us infer that the emulsion is made up of lean beef and bacon. The chemical composition of the raw materials is as follows: $W_M = 75\%$, $P_M = 21.4\%$, $W_F = 19\%$, $P_F = 6\%$.

$$\alpha\, 21.4 + \beta\, 6 \geq 10.5$$

$$\alpha\, 75.0 + \beta\, 10.0 + 100\gamma \leq 69.6 \qquad (a')$$

$$\gamma = 0.975 - \alpha - \beta$$

$$\alpha\, 75.0 + \beta\, 19.0 + 97.5 - 100\alpha - 100\beta \leq 69.5 - 25\alpha - 81\,\beta \leq -28 \qquad (b')$$

$$\alpha\, 75 + 97.5 - 100\alpha - 100\,\beta \leq 7.24 \cdot \alpha\, 21.4 - 179.9\alpha - 100\,\beta \leq -97.5 \qquad (d')$$

And finally

$$\alpha + \beta \leq 0.975 \qquad (c')$$

Taking (*a'*), (*b'*), (*c'*) and (*d'*) inequalities for equalities, the curves can be plotted in Fig. 1.

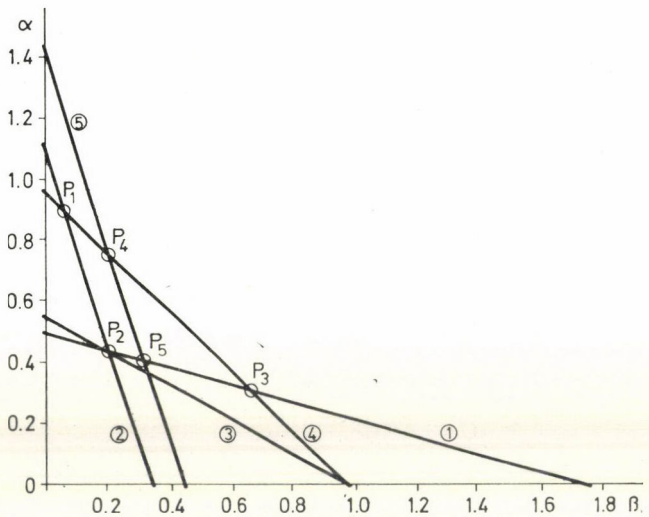

Fig. 1. Graphical model of linear programming. α = kg meat per kg raw emulsion; β = kg bacon per kg raw emulsion; P_1–P_5 = analysed intersections; ①–⑤ signs mean the (a)–(d) and (f) conditional equations and objective function in accordance with the text

It may be proven mathematically that it is sufficient to calculate the cost prices in the intersections and the composition of lowest cost is at the same time the minimum one (KREKÓ, 1959; CSÁTH, 1972). The beef was calculated at an average price of 71 Ft kg^{-1} and the bacon at 16 Ft kg^{-1}. All the possible combinations of composition, which satisfy the conditional equations fall in the P_1, P_2, P_3 triangle.

The coordinates of the different points and the pertinent prime costs are calculated by means of the objective function:

P_1	P_2	P_3
$\alpha = 0.88$	$\alpha = 0.43$	$\alpha = 0.31$
$\beta = 0.08$	$\beta = 0.22$	$\beta = 0.665$
$C = 63.75$ Ft kg^{-1}	$C = 34.05$ Ft kg^{-1}	$C = 32.65$ Ft kg^{-1}

1.3. Analysis of the solution of the equations

The composition under P_3 is the cheapest, *i.e.* if the emulsion is prepared only of meat and bacon without the addition of extraneous water. This is because the price of bacon is extremely low considering its protein content. Thus it follows that *point P_2 of maximum water content does not necessarily provide the minimum prime cost.* It is easy to prove that this occurs when

$$\frac{C_M}{C_F} > \frac{P_M}{P_F},$$

or the meat price : bacon price ratio is higher than the meat protein : bacon protein ratio (in this case 4.44 and 3.56, respectively). If the protein content of bacon is lower, *e.g.* 4%, composition P_2 of maximum water content gives the minimum prime cost.

The cost functions belonging to constant prime costs were not illustrated in Fig. 1, however, they may be easily plotted, since

$$\alpha = \frac{C}{C_M} - \frac{C_F}{C_M}\beta.$$

Further, it is evident that if the protein content of bacon were about 10% the minimum cost would be achieved by comminuting the bacon with the additives but without the addition of meat and extraneous water.

However, this would be an absurdity from the aspect of technology, thus the lower limit value of moisture content in fresh sausages must be also included in the conditional equations so as to ensure the character of the product. According to our present knowledge this is given as 62%:

$$\alpha W_M + \beta W_F + 100\gamma \geq 62 \qquad (f)$$

In the present example:

$$\alpha\, 75 + \beta\, 19 + 100\,(0.975 - \alpha - \beta) \geq 62 - 25\,\alpha - 81\,\beta \geq -35.5 \qquad (f')$$

Considering (f') inequality as equality the situation is illustrated in Fig. 1.

Because of the high α value the point P_4 cannot represent minimum cost, only point P_5 ($\alpha = 0.40$, $\beta = 0.32$, $C = 33.52$ Ft kg^{-1}). Thus this is the composition representing minimum cost ($\gamma = 0.975$–0.40–$0.32 = 0.253$).

Accordingly, 100 kg emulsion should be composed as follows:

lean beef	40.0 kg
bacon	32.0 kg
water	25.5 kg
additives (salt, seasoning, polyphosphate)	2.5 kg
Total	100.0 kg

For the sake of simplicity the calculations were related to 1 kg or 100 kg emulsion. Equation (*d'*), shown in Fig. 1 may now be neglected, since it is below points P_2 and P_5 representing maximum and minimum addition of water, respectively. This means that in this case only 75.6% water may be added instead of 80%, because of limiting condition (*b*).

1.4. Accounting for the difference in the composition of the emulsion and the final product

The treatment and storage of the fresh sausage involves loss of weight. This may be considered in the calculations as loss of water since the loss of solids is very low. However, the limit values for moisture and protein content, as specified in the standard, relate to the final product and not to the emulsion (MOCSÁRY *et al.*, 1969).

Accounting for percentage weight loss (L) the following simple material balance may be described between the moisture content of the emulsion and the final product (W_{MS} or W_E):

$$\frac{W_{MS} - L}{100 - L} \cdot 100 = W_E$$

and

$$W_{MS} = \frac{W_E}{100} \cdot (100 - L) + L.$$

Taking 2.5% for the average of industrial loss upon heat treatment and storage,

$$W_{MS} = \frac{69.5}{100} (100 - 2.5) + 1.5 = 70.26\%$$

if

$$L = 3.5\%$$
$$W_{MS} = 70.57\%.$$

Thus, the initial moisture content of the emulsion may be increased and accordingly the appropriate W_{MS} value is written on the right side of equation (*b*). On the other hand the protein content may be increased on account of the loss of moisture:

$$P_{MS} = P_E (100 - L)$$

where P_E is the protein content in the final product, P_{MS} is the protein content of the emulsion. If $L = 3.5\%$,

$$P_{MS} = 10.13\%$$

Thus, the appropriate P_{MS} value may be inscribed on the right side of equation (*a*). Thereby the program may be improved.

2. Expansion of the system of equations by accounting for dispersion

2.1. Analysis of the scatter arising from chemical composition

The comparatively simple calculations given above are complicated by the unalterable fact that the character of raw materials and of the final product shows scatter. In order to find a mathematical solution to this problem the nature of the scatter arising in the course of fresh sausage production has to be thoroughly and precisely analysed (OJTÓZY & ZUKÁL, 1969).

At present we are, unfortunately, very far from being able to analyse every raw material prepared for processing. This is due not only to the partial lack of rapid analytical methods but to severe problems in the organization of production and other problems very difficult to resolve. These problems are not discussed in this paper. However, it is essential to analyse production management from the aspect of scatter.

Let us assume, for the sake of simplicity, that for the manufacture of 200 kg of fresh sausages 80 kg of meat and 64 kg of bacon are separately prepared and the optimal formula should be established on the basis of chemical composition.

It is evident that the raw material prepared (80 kg meat and 64 kg bacon) has a true average moisture content and protein content (μ_w and μ_p). If it would be possible to establish this value exactly there would be no need to account for the scatter component. For, however heterogeneous the raw material related to one sample element (*e.g.* fat content) may be, after comminution and homogenization its heterogeneity is independent of the heterogeneity of the raw materials themselves.

The question arises as to why we have to account for the scatter in the chemical composition of the raw material? This is because the values μ_w and μ_p are not known but estimated from the sample through values of $\bar{X}_w$ and $\bar{X}_p$ by determination.

As every statistical estimate, this has also a certain standard error ($\sigma_{\bar{x}}$). Here the inevitable uncertainty of sampling is taken into account. However, it would be a mistake to accept the principle: heterogenous raw material = heterogenous product.

Let us see now how the standard error of sampling in the course of the chemical analysis of the raw material is transferred to the scatter of the composition of the emulsion. If the estimated protein and water contents of the emulsion are represented by P_{MS} and W_{MS}, respectively

$$P_{MS} = \alpha P_M + \beta P_F$$

$$W_{MS} = \alpha W_M + \beta W_F + 100\gamma$$

Since the values P_M, P_F, W_M and W_F are derived from laboratory measurements, these as well as P_{MS} and W_{MS} values are random variables. In accordance with the law of propagation of standard error:

$$P_{MS} = \sqrt{\alpha^2 \Delta P_M^2 + \beta^2 \Delta P_F^2}$$

$$W_{MS} = \sqrt{\alpha^2 \Delta W_M^2 + \beta^2 \Delta W_F^2},$$

where ΔP_M, ΔP_F, ΔW_M, ΔW_F represent the standard error in the protein and moisture contents of meat and bacon arising from taking random samples.

Some scatter remains in the chemical composition of the homogenized emulsion as related to the weight of one sample element. The more thorough the comminution and homogenization the lower is this value. This component of the standard error (marked $\sigma_{P,MS}$ and σ_W) has to be determined experimentally in relation to the weight of the sample element as specified in the standard.

The emulsion is filled into casings, it undergoes heat treatment and will be kept in cold storage prior to distribution. The loss of weight upon storage is different for individual sausages and for the batch. Since the chemical composition is affected by the weight loss, fluctuations in weight are reflected by fluctuations in the chemical composition.

In order to be able to account for this component of the standard error in a given plant the scatter of weight loss between batches (σ_t) and the scatter of weight loss of individual sausages (σ_r) within the batch has to be carefully measured.

In accordance with vectorial pooling of standard error the component originating from weight loss (σ_L) is as follows:

$$\sigma_L = \sqrt{\sigma_t^2 + \sigma_r^2}$$

The average weight loss must be accounted for on the right side of conditional equations (*a*) and (*b*), as mentioned earlier. The value σ_t has to be converted to water content or protein content on the basis of the relations shown below (given without demonstration):

$$\Delta w = \frac{100 - \overline{W}_{MS}}{(100 - \overline{L})^2} \cdot 100\, \sigma_L$$

where

ΔW standard error of the moisture content derived from the scatter of weight loss,

$\overline{W}_{MS}$ plant average of the initial moisture content of the emulsion,

$\overline{L}$ plant average of percentage weight loss.

$$\Delta P = \frac{\overline{P}_{MS} \cdot 100}{(100 - \overline{L})^2}\, \sigma_L$$

where

ΔP standard error in the protein content derived from the standard error of weight loss,

$\overline{P}_{MS}$ plant average of the initial protein content in the emulsion.

As shown earlier (KÖRMENDY, 1957), migration of the fat is observed in the sausage during heat treatment. Thereby we discovered that the fat content shows a tendency to increase from the suspension point downwards. This has been a significant factor in increasing scatter using the old way of sampling where the sample was taken by cutting the sausage perpendicularly to its length.

The cutting of the sausage length-wise, as proposed in the draft standard, would eliminate this component of scatter.

Scatter results from the method of determination, too. However, in the case of careful laboratory work this is negligible in relation to the other components of scatter. (In chemical analysis random errors are less dangerous than the systematic ones. The carrying out of parallel determinations serves primarily as an inner control against systematic errors rather than against random ones.)

Standard error in the final product (σ_p and σ_w) is calculated as follows:

$$\sigma_p^2 = \Delta P_s^2 + \sigma_{P,MS}^2 + \Delta P^2 =$$

Scatter upon sampling Heterogeneity of emulsion Uneven loss of weight

$$= \alpha^2 \Delta P_M^2 + \beta^2 \Delta P_F^2 + \sigma_{P,MS}^2 + \Delta P^2 \qquad (g)$$

$$\sigma_W^2 = \Delta W_S^2 + \sigma_{W,MS}^2 + \Delta W^2 = \alpha^2 \Delta W_M^2 + \beta^2 \Delta W_F^2 + \sigma_{W,MS}^2 + \Delta W^2 \qquad (h)$$

2.2. Including the standard errors in the conditional equations

Lately, the standard specifies different limit values for one sample and for the average of a four-element sample. In the case of fresh sausage the values pertaining to protein are 9.5% and 10.5%, resp. Assuming normal distribution and a probability level of 97.5%, that means:

$$\mu - \frac{2\sigma}{\sqrt{4}} \geq 10.5 .$$

Since $\frac{2\sigma}{\sqrt{4}} = \sigma$ and the statistical question is one-sided, the double scatter has to be built in the conditional equation.

In the material balance of protein the scatter is added to the value on the right side of the equation, while the value of the scatter of moisture content has to be subtracted in order to fulfil the standard specifications in an unfavourable case, too:

$$\alpha P_M + \beta P_F \geq P_N + 2\sigma_p \qquad (i)$$

$$\alpha W_M + \beta W_F + 100\gamma \leq W_N - 2\sigma_w \qquad (k)$$

where the values σ_p and σ_w are obtained by equations (g) and (h).

Since the values of σ_p and σ_w depend also on the value of α and β, the, correlations will not be linear, because, after the rearrangement of the equation α and β occur squared. In the protein balance for instance:

$$\alpha P_M + \beta P_F \geq P_{MS} + 2\sqrt{\alpha^2 \Delta P_M^2 + \beta^2 \Delta P_F^2 + \sigma_{P,MS}^2 + \Delta P^2} .$$

It is obvious that the numerical or graphical solution of such an intricate equation would require a fair amount of time, therefore two solutions present themselves: The intersections of the functions are determined by iteration. First the relation is calculated without standard error. Thus values α_1 and β_1 are obtained at the intersections. On the basis of these values, P_{MS} and W_{MS} are calculated. (The rest of the components of standard error are known from

previous determinations.) These are substituted in the equations and the coordinates of the intersections are calculated anew (α_2 and β_2). This process is continued until after two subsequent iteration steps the values differ very little. According to experience this occurs after two steps. The disadvantage of the process is that its correctness could not be unambigously proven mathematically so far. Only empirical results are available which seem to confirm that the process is convergent.

By means of a computer the exact solution may be obtained using the original equations. This is expedient, because the iteration operations require much time. Further the use of the original equations eliminates all mathematical scruples (KÖRMENDY & ERDŐS, 1977).

3. Conclusions

The model developed above correlates the chemical parameters of the raw materials used to the quality characteristics as specified in the standard. It takes into account the mathematical statistical aspects, as well. Thus it is suitable for the development of industrial production formulae.

Since, however, it permits also calculations with any hypothetical data, it enables the modelling of theoretical experiments as well (*e.g.* the change in the formula upon gradual modification of the composition of one of the components or of the scatter).

Data of prime cost supplement this kind of analysis by economic parameters, as it is always the prime cost of the actual amount of raw materials that occurs in the objective function.

Nomenclature

C	objective function, *i.e.* prime cost of the final product
C_F	prime cost of bacon
C_M	prime cost of the meat
Ft	forint
L	weight loss, percentage
$\bar{L}$	percentage weight loss as plant average
ΔP	scatter of the protein content as derived from the scatter of weight loss
P_E	protein content of the final product, %
F_F	protein content of the bacon, %
ΔP_F	standard error of the protein content of bacon
P_M	protein content of meat, %
ΔP_M	standard error of the protein content of meat
P_{MS}	protein content of the emulsion, %
ΔP_{MS}	standard error of the protein content of the emulsion
$\bar{P}_{MS}$	initial protein content of the emulsion, average per plant, %
P_N	protein content as specified in the standard, %
ΔW	standard error of the moisture content as derived from the scatter of weight loss
W_E	moisture content of the final product, %

W_F	moisture content of bacon, %
ΔW_F	standard error of the moisture content in bacon
W_M	moisture content of meat, %
ΔW_M	standard error of the moisture content of meat
W_{MS}	moisture content of the emulsion, %
ΔW_{MS}	standard error of the moisture content in the emulsion
$\overline{W}_{MS}$	plant average of the initial moisture content in the emulsion, %
W_M	moisture content as specified in the standard, %
$\overline{X}_P$	estimated average protein content of the raw materials
$\overline{X}_w$	estimated average moisture content of the raw materials
α	kg meat per kg raw emulsion
α_1	the value of α obtained by the first iteration
α_2	the value of α obtained by the second iteration
β	kg bacon per kg raw emulsion
γ	kg added water per kg raw emulsion
μ_p	true average of the protein content in the raw materials
μ_w	true average of the moisture content in the raw materials
σ_L	the component of scatter originating from the weight loss
σ_p	scatter of the protein content in the product
$\sigma_{p,MS}$	scatter of the protein content in the homogenized emulsion
σ_r	standard error of weight loss within one batch
σ_t	standard error of weight loss between batches
σ_w	standard error of the moisture content in the product
$\sigma_{w\,MS}$	standard error of the moisture content in the homogenized emulsion
$\sigma_{\bar{x}}$	standard error

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PRODUCTION OF A FATTY ACID UREA ADDUCT AND ITS APPLICABILITY IN THE FEEDING OF RUMINANTS

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For the feeding of ruminants an energy-rich, synthetic, native protein substitute based on an inorganic nitrogen source has been developed. This product is the fatty acid urea adduct.

Investigation was made into the adductability by urea of fatty acids with different carbon chain length and saturation used as the energy source. It has been established that the adductability of saturated fatty acids with carbon numbers in the range of 12–22 is excellent and it decreases as a function of the extent of their unsaturation.

A large-scale, industrial level technology has been developed for the manufacture of the fatty acid urea adduct; this technology is patented. The rationale of the process: the adductive reaction takes place in an aqueous medium, and the water is removed from the fatty acid urea adduct by cold powdering. The process leads to a dust–like, free-flowing fatty acid urea adduct as the end product.

The adduct content of the fatty acid urea preparation may be determined by the DSC method. The extent of hydrolysis in the product was established by means of *in vitro* and *in vivo* methods; its hydrolysis is much more protracted than in the case of urea itself. Experiments *in vivo* supported and confirmed those performed *in vitro* because:

– urea and ammonia level in the rumen and blood can be detected over a much longer period from intake than in the case of urea substance and the concentrations gave also favourable values with toxic hazards being ruled out;

– the amount of urea contained in 0.8 g adduct per kg body weight when fed to sheep, involved no detectable alterations either in clinical symptoms or in laboratory tests for toxic effects. While, on the other hand, an urea application ratio of 0.8 g kg^{-1} body weight for sheeps invariably led to poisoning which frequently turned out to be lethal.

We formulated recipes for the applicability of fatty acid urea adduct product in the feeding of ruminants of different ages and breeds. On the basis of these recipes, feeding experiments were carried out by the NATIONAL FEEDSTUFF INSPECTORATE (OTEF) and as a result of these investigations a permission for the manufacture and marketing of this product was issued.

There is a world-wide endeavour to apply synthetically produced nitrogen sources as substitutes for protein. The primary aim of our research work was to develop a compound which enables a part of native proteins used in ruminant feeding to be replaced by synthetic, nitrogen-containing feeds without toxic hazards and, at the same time, to provide a low-cost energy carrier.

The energy-rich protein substitute was made of a mixture of distilled animal and vegetable fatty acids and urea. The decomposed soap precipitate, which is a by-product in oil refinery, may also be used as a fatty acid source, whereby the problem of by-product utilization is also adequately resolved.

The decomposed soap stock can be adducted with urea even at a triglyceride concentration of 50% and free fatty acid content of 50%. In this case the consistency of the adduct is greasy. From the fully hydrolysed and distilled soap stock fatty acids a powdered product may be prepared if allowance is made for a proper fatty acid: urea ratio (40: 60–45: 55% by weight). This product ensures an easy admixture of fatty acids to feedstuffs and, at the same time, the urea loses thereby its inconvenient hygroscopic property.

In the molecular structure of the fatty acid urea adduct, the fatty acids are located within the molecular lattice of urea. This structure protects fatty acids against atmospheric oxygen and prevents peroxide formation. The solubility of urea in the fatty acid urea adduct structure is worse than that of solid urea. As a result of this retarding effect there is a better possibility for the ruminal microflora to synthesize ammonia released from urea into protein than in the case of solid urea.

In view of its high starch value and N content, the fatty acid urea adduct powder is supplied to the mixing plant as industrial concentrate, from which further concentrates and grain feed are produced for ruminant livestock of different breeds and ages.

The adduct-forming ability of urea may be explained by the special structure of its molecule. Urea may be classed into the group of inclusion-forming compounds (BENGEN, 1951; SCHLENK, 1949, 1950a, b). The urea molecules attached to each other by hydrogen bonds are located along spirals. Within the spirals there are empty channels inside. In these channels, alien molecules of proper size can be lodged, accompanied by the formation of non-stoichiometrical inclusion compounds. The internal cross section of these channels is of the order 5 Å. Therefore, there is a possibility for the inclusion of *e.g.*: fatty acids or other long chain molecules (SCHLENK, 1949, 1952). Experimental experiences indicate that adduct formation takes place with straight chain compounds and not with ones containing aromatic rings (ZIMMERSCHIED, 1950; SMITH, 1952). Adduct formation is limited by chain length and, in case of various organic compounds, by the character of the functional group. The lowest carbon number fatty acid to form adduct with urea is capronic acid (HERMANN, 1952). Observations show that adduct formation takes place without difficulty even with molecules of as many carbon atoms as 50 but a theoretical upper limit is imposed by hydrocarbons with 55 carbon atoms (HOLUSEK & IBRAHIM, 1953; SCHLENK, 1951; NEWEY, 1950; RIGAMONTI & RICCIO, 1952, 1953).

Adduct formation is an equilibrium process. The equilibrium state is a function of temperature, concentration of urea and adduct-forming components and of the type of solvent. Other things being equal, the stability of additioned urea compounds is directly proportional to the molecular weight of the coupled components. The number of unsaturated bonds in the compounds to be adduct-

ed affects adduct formation. With increasing unsaturation, adduct formation becomes increasingly difficult or impossible. This is explained by the fact that unsaturated bonds in the chain involve changes in orientation, which presents obstacles in the insertion and adjustment to the system of urea molecules. Most urea adducts get dissociated at rising temperatures. In addition to the components to be adducted, adduct formation is promoted by certain materials, *e.g.* solvents, and inhibited by others. The most widely used solvents applied to promote adduct formation are methanol, alcohols, ethers, ketones, and esters. The effect of solvents is explained by their ensuring the surface cleanness of urea crystals as urea solvents, whereby they promote penetration of organic adduct components into the urea skeleton. The chemicals inhibiting adduct formation (sulphur compounds and peroxides, *etc.*) exert this effect by their adsorption on the surface of the urea molecule and thus they prevent the integration of organic compounds into the crystalline structure of the urea molecule.

The adduct compounds of urea are of crystalline structure. The crystals may extend in length from a few mm to several cm. Since the composition of adducts does not follow the rule of molecular proportions but the amount of urea bound per molecule rises with the molecular weight of the organic substance to be adducted, spatial relations and structure may be assumed to have a decisive role in the structure of adduct crystals.

Between the urea and the organic component there is no connection resulting from chemical reaction; the forces cementing together the compound are of the order of the *Van der Waals* forces. Adduct formation is an exothermic process.

Adduct formation in fat chemistry is commonly used to separate fatty acids of various levels of saturation and carbon chain lengths for analytical and preparative purposes alike. In these procedures different organic solvents are applied, the role of which is as follows:

- they act as activators in adduct formation,
- they reduce the viscosity of the adduct formed, which facilitates easier handling,
- they reduce the reaction threshold of different phases of the components to be adducted,
- by these solvents the non-adducted components may be washed out.

The primary objective of our investigations was to analyse the possibility to adduct fatty acids with urea in aqueous medium.

Materials and methods

The velocity and quantitative relations of fatty acid urea adduct formation in aqueous medium were studied with particular reference to, and emphasis on, the material properties of the fatty acid mixture used for the addition and the effect of external factors under the process of adduct formation (such as fatty acid: urea ratio, mixing intensity, temperature and water content). The measure of adductability – with varying ratios of fatty acids to urea – for fatty acids of different chain length and level of saturation was determined on the basis of the composition of the fatty acid mixture to be adducted and the non-adducted part, with due allowance for the molar fraction of fatty acids related to urea.

For want of large amounts of pure, model fatty acids, industrial fatty acid mixtures were used which contained typical chain lengths and saturation levels. These mixtures are as follows:

- stearic acid mixture: featured by containing 92% saturated fatty acids of carbon numbers C_{16}–C_{18},
- palm-kernel fatty acid mixtures of shorter carbon chain: contains 60% fatty acids of C_{12}–C_{14} carbon number,
- tallow fatty acid mixture: it contains 50% fatty acids of the types $C_{18:0}$ and $C_{18:1}$,
- less saturated fatty acid mixture with 20% doubly unsaturated C_{18} fatty acids as well,
- longer carbon chain length fatty acid mixture: rape-seed fatty acid mixture which contains 50% $C_{22:1}$ unsaturated fatty acid.

To find out if the fatty acid urea adduct was in fact an adduct, an instrumental, thermoanalytical method was applied; the instrument was the so-called dynamic microcalorimetric device (DSC). The method is based on the decomposition of the adduct sample into its component compounds upon heating to a certain temperature and the temperature of decomposition may be determined from the endothermic peak which appears on the thermogram. Since the decomposition temperature of the adduct (121 °C) is lower than the melting point of urea (128 °C), the endothermic peak indicating phase transition in urea can thus be distinguished from the decomposition of the adduct. The other component of the fatty acid urea adduct, the free fatty acid also changes phase at proper temperature but this being an endothermic process, it can be observed and traced on the thermogram. In view of the fact that the slip-point of fatty acids and the decomposition temperature of the adduct are widely separated, the presence of non-adducted free fatty acids in the adduct is also easy to detect.

In vitro experiments were made to establish the possibility and degree of hydrolysis and solubility of adduct urea embedded in granulated concentrate (pelleted feed) and containing fatty acid urea adduct. In our *in vitro* experiments 0.1% urea was introduced into a reaction mixture containing a phosphate buffer of 0.05 M and pH 6.8 and urease enzyme. The urea was dissolved and hydrolyzed at 37 °C, under intensive mixing. The extent of hydrolysis was determined by titrating samples taken every 20 minutes with 0.1 N HCl. Under similar conditions, the degree of hydrolysis in urea was also determined.

In the *in vivo* experiments acute toxicity tests were performed on sheep (25–31 kg) with fistula in their rumen by the application of pelleted feed concentrate prepared with fatty acid urea adduct. The test animals were individually given a mixed feed ration of 250 g with 16.3% crude protein content. For each kg of body weight 0.3, 0.6, 0.8 g urea in adduct form were applied to the animals *via* a rumen fistula in the form of granulated feed containing fatty acid urea. The experiments were made on four head of sheep. Subsequent to the application of the adduct-containing feed concentrate we investigated variations in pH of the rumen fluid, in concentrations of ammonia and urea in the rumen, and in the volatile fatty acid concentration of the rumen fluid as well as in blood urea and ammonia. The analytical methods applied were:

- pH value of the rumen fluid was determined by an electrometric method,
- ammonia content in the rumen fluid and blood by microdiffusion,
- urea content by a photometric method via the para dimethylbenzaldehyde reaction,
- volatile fatty acid content in the rumen by gas chromatographic method.

Samples from the rumen fluid and from blood were taken at every hour over eight hours counted from the introduction of the feed concentrate containing fatty acid urea adduct. Analytical data from the rumen content and blood of animals starved for 12 h and provided with water alone were considered as control values.

Results and conclusions

Figure 1 illustrates the adductability of fatty acids with different carbon chains and saturation at varying fatty acid urea ratios.

It appears from data in the Figure that the results of adduction experiments conducted in an aqueous medium are in agreement with literature data for similar experiments performed in organic solvents, that is, the adduct-

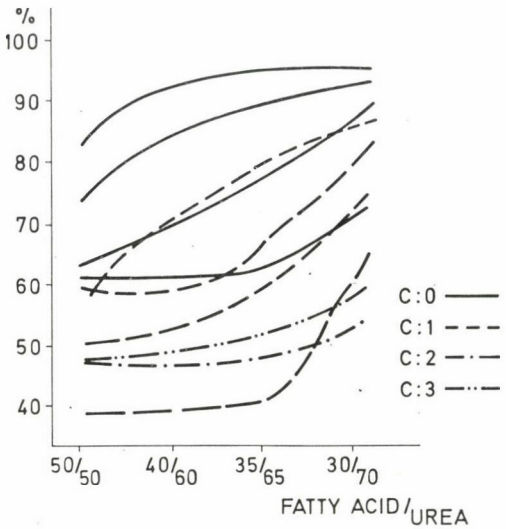

Fig. 1. Percentage of adducted fatty acids in fatty acid mixtures. Lines, in sequence, from the top; C:O ——— C 18:0, C 16:0, C 14:0, C 12:0; C:1 — — — C 22:1, C 20:1, C 18:1, C 16:1; C:2 —·— C 18:2; C:3 —··— C 18:3

ability of fatty acids rises in direct proportion with the increasing number of carbon atoms. By examining the amount of fatty acids with different numbers of carbon atoms as a function of the fatty acid urea ratio, it may be stated that by increasing the proportion of urea, the adducted amount of various fatty acids invariably grows but its intensity is directly proportional to the length of the fatty acid chain and inversely to the degree of its saturation.

The set of curves in Fig. 2 provides a combined plot for the dependence of the adductability upon the fatty acid urea ratio applied, the degree of saturation in fatty acids and the number of carbon atoms contained in them.

It follows from the linearity of the correlation that, given the fatty acid composition of a certain fatty acid mixture, the expected adduction percentage for a given fatty acid urea ratio may be determined by means of the curves. In addition to this practically useful correlation, the fact that the slope of the curves increases with increasing carbon chain length and with the degree of saturation allows inferences to be made about differences experienced in the propensity of fatty acids for adduct formation. These differences are likely to be the result of differences in the collisional and hit probabilities between urea and fatty acids of divergent structural patterns. The assumption appears to be unambiguous as regards the saturation of the carbon chain, because the spatial structure of unsaturated fatty acids hampers the addition of urea. Likewise, the lower affinity of fatty acids with shorter carbon chains might also be explained by a reduced hit probability. These assumptions appear to be supported by the fact that, in case of a higher urea fatty acid

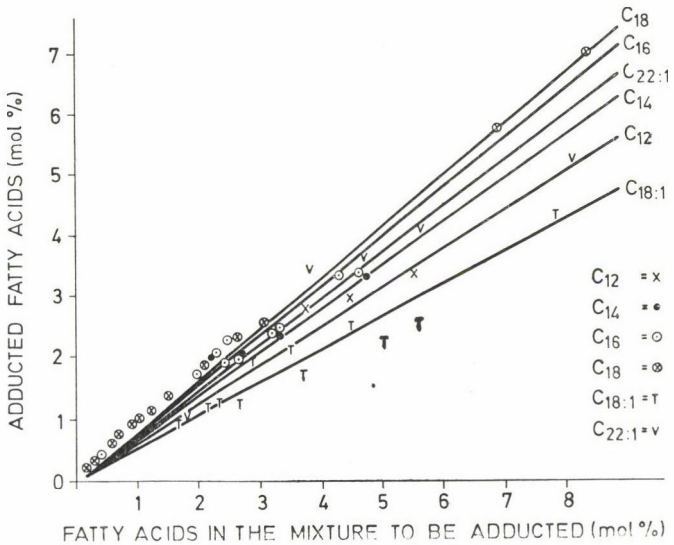

Fig. 2. Dependence of the percentual molar concentration of the adducted fatty acid mixture on that of the starting mixture

ratio, the probability of addition rises on account of the higher amount of urea, and the degree of adductability for fatty acid mixtures with different carbon numbers and saturations thus exceeds 90%.

Results of laboratory experiments convinced us that, in an aqueous medium, fatty acid mixtures of animal and vegetable origin can be completely adducted with urea. In case of specified quantitative proportions, a distilled mixture of plant and animal fatty acids and urea result in a powdery product. Accordingly, we designed and constructed an experimental pilot plant where 1000–1500 kg h^{-1} fatty acid urea adduct powder may be produced by continuous technology. This technology is patented; it consists briefly of the following major steps:

The aqueous urea solution is heated to a given temperature and dissolved and mixed with a distilled fatty acid mixture at a certain temperature in a so-called reactor tube, with a view to establishing active contact between the two components; the structure of the adduct is thus formed. In our technology the ratio of fatty acid to urea is 40 : 60. The titre of the distilled fatty acid mixture is 30 : 38. Excessive amounts of water are removed from the system by powdering in cold air. The powdered fatty acid urea adduct is then filled into sacks of 35–40 kg unit weight and transported to feed mixing and milling plants.

Figure 3 illustrates the dynamic thermoanalytical pattern for the urea adduct of distilled fatty acid mixtures of plant and animal origin.

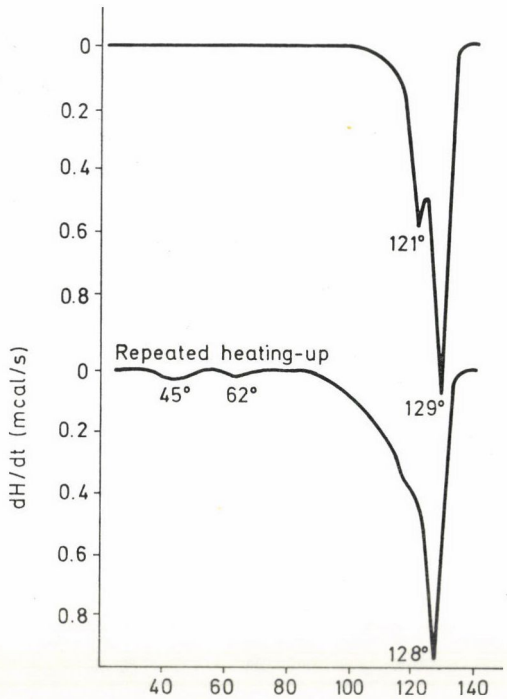

Fig. 3. Dynamic thermoanalytical pattern for the fatty acid urea adduct

The presence of free fatty acids can not be detected at all on the upper curve and the material investigated may therefore be considered as a 100% adduct. The lower curve stands for a product which had already been heated, that is split and then cooled. The diagram suggests that in this case most of the fatty acids is already free and at a given temperature (45 °C) an endothermic peak is visible indicating phase transition and the presence of free fatty acids.

These measurements help to determine the adduct character of the samples under study and the presence of free fatty acids. This provides a basis to establish the ratio of adducted and free fatty acids by calibration, *i.e.*, the degree of adductability of the adduct in question.

Figure 4 represents the curve of the rate of hydrolysis for urea in the fatty acid urea adduct and for substantial urea.

The extent of hydrolysis is expressed as a percentage of the total ammonia content of the urea introduced into the enzymatic system. Data in the Figure indicate that the hydrolysis of urea in the fatty acid urea adduct is characterised by uniformity and regularity while the hydrolysis of substantial urea is rapid and the total amount is hydrolysed in 40 min.

In Fig. 5 variations in the pH value of the rumen fluid of animals fed different amounts of fatty acid urea adduct are plotted against the time elapsed from the application of the test feed.

Prior to test feeding the pH value of the rumen fluid sample was slightly acidic (pH 7.2–6.5). Four to six hours after the intake of the test feed the acidity value decreases for all three urea rations. This phenomenon is of great

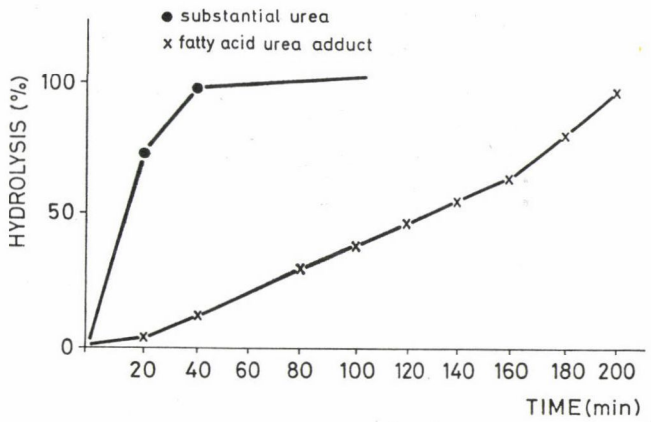

Fig. 4. *In vitro* hydrolysis of urea in fatty acid urea adduct

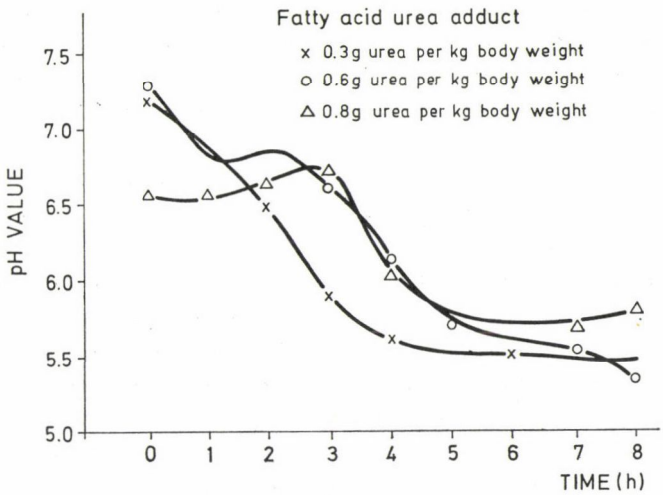

Fig. 5. Variations in the pH value of the rumen fluid with different concentrations of fatty acid urea adduct

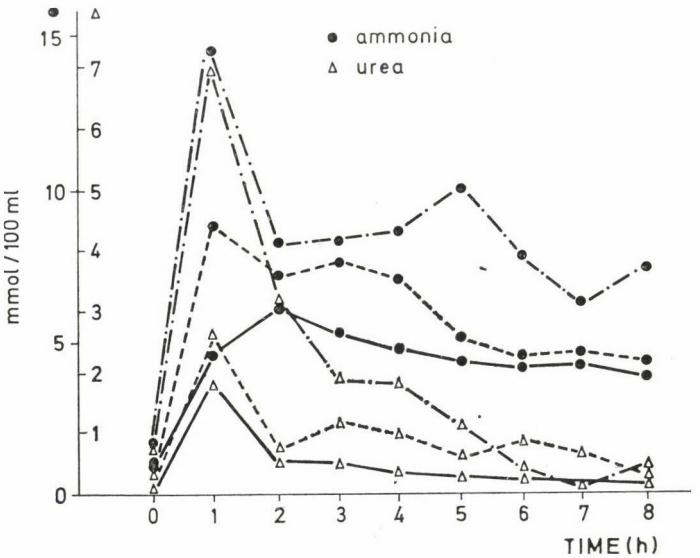

Fig. 6. Variations in the concentrations of ammonia and urea in the rumen fluid with different amounts of fatty acid urea adduct

–·–·–·–·–	ammonia with 0.3 g fatty acid urea adduct per kg body weight
●–––●–––●	ammonia with 0.6 g fatty acid urea adduct per kg body weight
●–·–●–·–●	ammonia with 0.8 g fatty acid urea adduct per kg body weight
–△–△–△–△–	urea with 0.3 g fatty acid urea adduct per kg body weight
–––△–––△–––	urea with 0.6 g fatty acid urea adduct per kg body weight
△–·–△–·–△	urea with 0.8 g fatty acid urea adduct per kg body weight

significance after urea intake because, on the one hand, it indicates that in spite of urea intake, fermentation in the rumen goes on normally, on the other hand, this means that there is no toxic hazard, the absorption of ammonia in the rumen is insignificant, and its use by the microflora in protein synthesis is optimal.

Figure 6 illustrates the variation of ammonia and urea concentrations in the rumen fluid as expressed in μmol per 100 ml, as a function of time elapsed from the application of the feed concentrate containing fatty acid urea adduct.

For all three urea doses, the ammonia concentration in the rumen fluid reaches its highest value between the first and second hour after feed application. Its concentration, however, failed to reach levels which might be termed as toxic. Subsequently, the presence of ammonia in the rumen is very protracted and it is higher than the control value even at the end of the eighth hour. With due attention paid to urea concentration in the rumen fluid and its change, it may be established that its hydrolysis is very much protracted and even at the 6–7th h after application, appreciable amounts of urea may be detected. It is widely known from literature data that substantial urea is

completely hydrolysed after 2–3 hours. In the case of fatty acid urea adduct the period of hydrolysis has doubled.

Figure 7 represents urea and ammonia concentrations in blood as a function of time.

The ammonia concentration of blood is plotted in units of μmol ammonia per 100 ml, that of urea in units of μmol urea per 100 ml blood.

The ammonia content of blood increased to 2–3 times the control value 1–2 h after the introduction or intake of urea adduct. But even this value is far below the toxic concentration. In case of the application of the same amount of substantial urea it may even attain the value of 80–100 μmol per 100 ml. After the second hour counted from the time of application, the ammonia concentration in the blood tends to slowly drop but its presence is protracted. This phenomenon is contrary to those experienced in toxic cases. The urea content in blood starts to rise 2–3 h after application, then it remains at a constant level for a long time but its value is not high. This urea concentration pattern in the blood indicates its good utilization by microorganisms in the rumen and in the internal metabolism. In toxic cases the urea concentration in blood is higher and it tends to rise gradually.

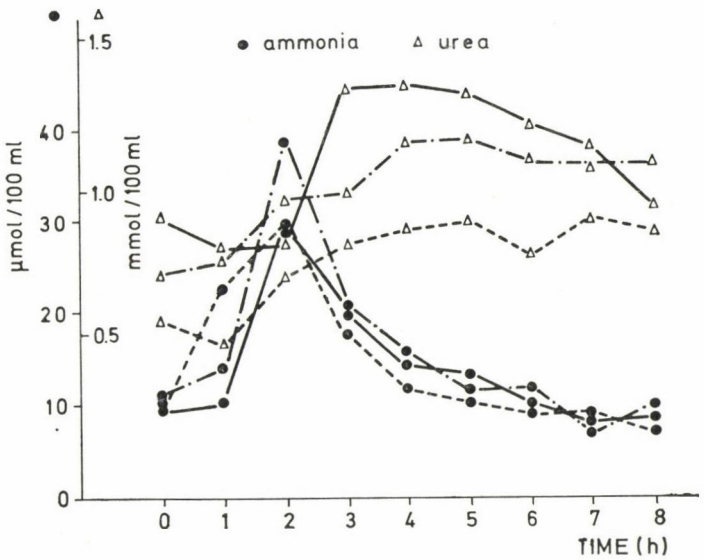

Fig. 7. Variations in the ammonia and urea concentrations in blood with different amounts of fatty acid urea adduct

−·−·−·−·− ammonia with 0.3 g fatty acid urea adduct per kg body weight
−−−−●−−−−● ammonia with 0.6 g fatty acid urea adduct per kg body weight
−·−●−·−● ammonia with 0.8 g fatty acid urea adduct per kg body weight
△−△−△−△− urea with 0.3 g fatty acid urea adduct per kg body weight
△−−−△−−−△ urea with 0.6 g fatty acid urea adduct per kg body weight
△−·−△−·−△ urea with 0.8 g fatty acid urea adduct per kg body weight

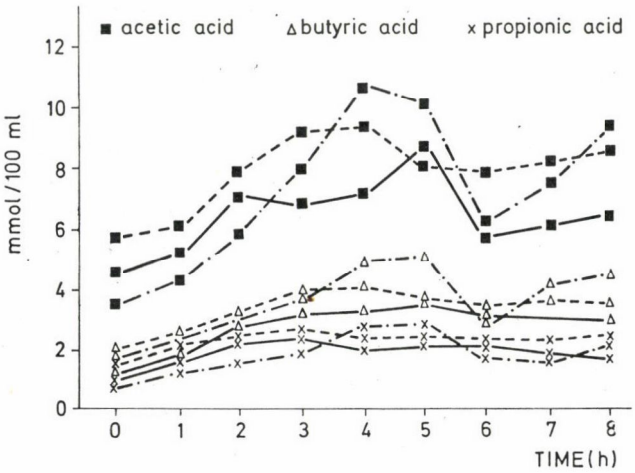

Fig. 8. Volatile fatty acids in the rumen fluid

■–■–■–■ acetic acid with 0.3 g fatty acid urea adduct per kg body weight
■---■---■ acetic acid with 0.6 g fatty acid urea adduct per kg body weight
■–·–■–·–■ acetic acid with 0.8 g fatty acid urea adduct per kg body weight
△–△–△–△–△ propionic acid with 0.3 g fatty acid urea adduct per kg body weight
△---△---△ propionic acid with 0.6 g fatty acid urea adduct per kg body weight
△–·–△–·–△ propionic acid with 0.8 g fatty acid urea adduct per kg body weight
×–×–×–× butyric acid with 0.3 g fatty acid urea adduct per kg body weight
×---×---× butyric acid with 0.6 g fatty acid urea adduct per kg body weight
×–·–×–·–× butyric acid with 0.8 g fatty acid urea adduct per kg body weight

Figure 8 shows the time function of the volatile fatty acid content in the rumen fluid, expressed as molar concentration.

The total molar concentration of volatile fatty acids keeps on rising from the time of introduction and reaches a maximum in the 4–5th hour. This indicates that fermentation in the rumen has a normal course, *i.e.*, it is not disturbed, it is in fact promoted by the fatty acid urea adduct. The molar concentration of all three volatile fatty acids (acetic, propionic and butyric acids) is steadily and definitely rising until the 4–5th hour counted from application. Their mutual proportion and percentual distribution suffers no substantial alterations under the effect of the fatty acid urea adduct. This proportion in general corresponds to 57–58% acetic acid, 25–28% propionic acid and to 14–18% butyric acid. Numerical results obtained for the total amount of volatile fatty acids and their mutual ratio provide substantial evidence in favour of the possibility to feed livestock with fatty acid urea adducts because an improper application of urea may exert harmful effects partly on the formation of volatile fatty acids, and may affect their quantitative distribution. Occasionally it augments the concentration of undesirable butyric acid.

In vivo experiments indicate therefore that urea in the adduct undergoes a protracted hydrolysis; as a result of this long hydrolytic process, the application to sheep, with rumen fistula, of 0.8 g kg^{-1} body weight urea adduct did not lead to harmful effects detectable by clinical symptoms or by toxic effects in laboratory tests. On the other hand, in case of substantial urea a dose of 0.8 g kg^{-1} body weight has invariably led to poisoning in sheep with frequent lethality.

Based on findings from these experiments we consider that, with the adduct, a larger proportion of the crude protein requirement of ruminants may safely be covered than with substantial urea.

Given the above experimental results, we have formulated recipes for feedstuffs containing fatty acid urea adduct and conducted feeding tests in cooperation with the NATIONAL FEEDSTUFF INSPECTORATE (OTEF) with ruminants of different breeds and ages. Compositional features for these recipes and the results of feeding experiments are briefly reviewed here:

Adduct-containing starter feed for calves:

starch value: 74 kg per 100 kg

crude protein: 20%

distribution of crude protein: 23% from NPN and 77% from native protein; this starter feed with adduct may be fed from 10 days of age *ad libitum*, together with skimmed milk and an addition of alfalfa and hay up to the age of 60 days.

Adduct-containing calf-rearing feed:

starch value: 76 kg per 100 kg

crude protein: 19%

crude protein distribution: 36% NPN, 64% native protein. This may be applied *ad libitum* from 60 to 150–180 days of age. Between the age of 10 to 150–180 days, our experiments indicated a daily weight gain of 1000–1050 g on the average.

Adduct-containing concentrate for beaf cattle:

starch value: 67 kg per 100 kg

crude protein: 42%

crude protein distribution: 68% NPN, 32% native protein.

This feed may be used in the grain feed-based fattening technology from the weight of 150–180 kg. The concentrate is added to daily ground corn portions in the proportion of 10–12% 0.7–0.9 kg/day/animal. Large-scale stock farms in Hungary have been using the adduct-supplemented concentrate with beef cattle for five years. Twentyfive to thirty thousand head of finished cattle are marketed every year. The average daily weight gain over the whole fattening period (up to a finishing live-weight of 150–600 kg) is 1250–1400 g

per day. The average carcase weight is 63.5%. Feed costs per one kg liveweight at 18–20 Ft.

Adduct-containing feed for dairy cows:

starch value: 75 kg per 100 kg
crude protein: 19%
distribution of crude protein: 40% NPN, 60% native protein.

Above a milk yield of 8 liters, 0.4 kg of feed is applied on every liter. It keeps the milk yield of cows at a steady level and increases fat content in milk by 0.01–0.02%. The production cost of one liter milk is cheaper by 0.95–1.2 Ft than with other dairy cow feeds.

Uniform, adduct-containing feed for ewe and starter and fattening feed for lambs:

starch value: 67 kg per 100 kg
crude protein: 17%
crude protein distribution: 34% NPN and 66% native protein.

The advantage of this feed for sheep lies in the fact that the same standard composition may equally be used for in-lamb ewes, suckling and fattened lambs.

This set of recipes may be used for individual cattle of different age and sex. In our view, its advantage is that the rumen microflora is already made capable of urea utilization from as early as the age of 10 days. This composition is suitable to replace native protein to a large extent with success and safety. On the basis of feeding tests and large-scale feeding experiments its application proved to be economical.

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KINETIC ANALYSIS OF CONTINUOUS YOGHURT FERMENTATION

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After a thorough study of batch fermentation continuous yoghurt fermentation was realized by converting inoculum production into a continuous process. On the basis of evaluation of the specific growth rate coefficients of batch fermentations, experiments were made in the range of dilution rates between 0 and 1.5 h^{-1} at 45 °C for devising the continuous process. The inoculum obtained in a continuous process was used to prepare yoghurt and the product was compared with yoghurt available on the market and was proved to be of identical quality.

When inoculum produced by the continuous process was used for yoghurt manufacture the incubation period was found to be 10–15% shorter than in traditional processing. The incubation period of yoghurt of 36 °SH was 135 min, on the average.

In the first part of this study (REICHART, 1978) the technology of yoghurt manufacture was described and the possibility and aspects of converting the process into a continuous one were analysed in detail.

In order to determine the parameters of continuous processing, the kinetic constants of batch fermentation have to be determined. Experiments were carried out to establish the optimum temperature and the optimum amount of inoculum. The specific growth rate coefficients (k) of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, constituting the microflora of yoghurt, was determined at different temperatures. It was found that, in identical fermentation, the k value of *Str. thermophilus* between 318 K (45 °C) and 323 K (50 °C) was significantly higher than that of *Lb. bulgaricus*. The growth rate of both strains was highest at 318 K (45 °C). In fermentations at 323 K (50 °C), signs of degeneration appear and thus sound propagation cannot be expected. In fermentations started with 3 or 5% inoculum, no significant difference was observed in the k values. However, in fermentations started with 1% inoculum, diauxie was observed in almost every case leading to uncertainties in the planning of dilution rate.

On the basis of data reported earlier, the use of a starter culture of 3% inoculum at 318 K was found expedient for the induction of a continuous fermentation.

In case it is the inoculum fermentation and not the main fermentation that is made continuous as suggested by other authors (SCHULER, 1971;

LELIEVELD, 1976), the danger in wasting the total amount of milk due to fluctuations of the rate of dilution can be evaded.

In determining the applicable rate of dilution, the 95% confidence interval of specific growth rate coefficients was taken into account. The coefficients at 318 K (45 °C) are as follows:

for *Str. thermophilus*, $k = 0.0364 \pm 0.0068$ min^{-1}

for *Lb. bulgaricus*, $k = 0.0284 \pm 0.0068$ min^{-1}.

As shown above the k value belonging to *Lb. bulgaricus* is significantly lower than that of *Str. thermophilus*, thus the applicable dilution rate is limited by the lower confidence limit of the k value belonging to *Lb. bulgaricus*. Consequently, the upper limit of the dilution rate (D), where the safety of avoiding washing out is 95%, equals 0.0216 min^{-1}. In the course of the experiments a somewhat higher dilution rate, $D = 0.025$ min^{-1} (1.50 per h), was also applied and washing out was not observed.

The experiments were carried out at 318 K (45 °C) with an inoculum of 3% at dilution rates of 0, 0.6, 1.2 and 1.5 h^{-1}, resp., in 2 replicates each. The inoculum produced in a continuous process was added to milk in plastic containers and this was fermented into yoghurt. The sensory evaluation of the product was carried out by the plant inspectors of the DAIRY CONTROL STATION, TRUST OF DAIRY ENTERPRISES, Budapest. The time requirement of producing yoghurt of a uniform acidity of 36 °SH was also determined.

1. Materials and methods

1.1. Yoghurt culture

The culture used in the experiments was a mixed culture of *Str. thermophilus* and *Lb. bulgaricus*, obtained from the DAIRY CONTROL STATION. The culture was maintained by subculturing weekly into sterile milk. It was incubated at 318 K (45 °C) for 2–2.5 h and stored at +5 °C.

1.2. Milk used for yoghurt production

The milk used in the experiments was devoid of inhibiting agents, it was fresh cow's milk, heated prior to use in flowing steam for 30 min and cooled to the appropriate temperature.

1.3. Experimental equipment

The same basic equipment was used as in the batch fermentation experiments (REICHART, 1978) and this was complemented for continuous use. The equipment is shown in Fig. 1.

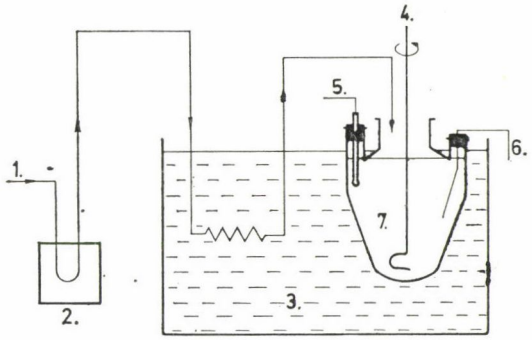

Fig. 1. Scheme of the experimental yoghurt fermentation equipment. 1. Milk pipes; 2. peristaltic feeding pump; 3. Wassermann water bath; 4. stirrer; 5. electrode for pH measurement; 6. overflow pipe of fermentor; 7. fermentor

In order to ensure 1400 ml useful volume, an overflow level adjuster was used. The required level was determined by the height of the orifice of the outlet pipe. The required volume rates were set by the application of a S–31 type peristaltic feeding pump. The sample required for acid titration or cell counting was taken at the orifice of the overflow pipe.

1.4. pH measurement

A SEYBOLD GPB type apparatus with immersed combined glass electrode was used to measure pH.

1.5. Acid titration

The *Soxhlet-Henkel* procedure, generally used in the dairy industry, was applied. The degree of acidity (°SH) is understood to mean the number of ml of 0.25 N NaOH required to neutralize 100 ml milk in the presence of phenolphthalein. In practice 20 ml milk are titrated in the presence of 0.2 ml alcoholic phenolphthalein solution to light rose with a 0.1 N NaOH solution. The degree of acidity is obtained by multiplying the number of ml of the alkali solution used, by 2.

1.6. Determination of cell count

Utilizing the difference in the morphology of the two bacteria, cell count was determined according to *Breed* (DEMETER, 1967). Below the iso-electric point of casein, pH = 4.7, this determination becomes uncertain. Thus in this range only the ratio of the cocci to rods was studied.

1.7. Preparation of yoghurt

Having reached the steady state at the various dilution rates, 200 ml of milk, set at 318 K (45 °C), prepared for continuous fermentation, were incubated with 3% culture. Inoculation and subsequent incubation were carried out in plastic containers, used in the industry. Thus they were comparable with the commercial product filled in similar containers.

1.8. Determination of the incubation period

The time required for clotting the milk set at 318 K (45 °C) and incubated with cultures prepared at various dilution rates, may be determined relatively accurately by the appearence of a narrow strip of whey of a few mm. After observation of clotting the containers were incubated for another 30–40 min in a water bath and then cooled to 5 °C in a refrigerator.

In order to be able to compare products of identical consistency and degree of acidity, the incubation periods were uniformly converted into the period required to reach 36 °SH by means of the following empirical equation:

$$t_{36} = t_{\text{measured}} + \frac{36 - {}^{\circ}\text{SH}_{\text{measured}}}{0.3} \text{ (min)}$$

where

t_{36}	is the time required to reach 36 °SH (min)
t_{measured}	is the actual incubation period (min)
$^{\circ}\text{SH}_{\text{measured}}$	is the degree of acidity of the product
0.3	is an empirical constant, rate of increase in degree of acidity ($^{\circ}\text{SH min}^{-1}$).

The value 0.3 $^{\circ}\text{SH min}^{-1}$ is valid for the final phase of the exponential growth period of *Str. thermophilus* in traditional, non-agitated fermentations at 318 K (45 °C). Because of the thermal capacity of the product, some increase in the degree of acidity has to be reckoned with, after placing it in the refrigerator. Assuming a rate of 0.3 $^{\circ}\text{SH min}^{-1}$, the time required to reach 36 °SH at clotting:

$$\frac{36 - 25}{0.3} = 36.7 \text{ min}.$$

The correctness of empirical factor 0.3 was proven by the fact that after an incubation period of 30–40 min the grade of acidity of the samples kept in the refrigerator overnight reached the value of 36 within 1–2 °SH.

It should be noted, however, that the factor 0.3 $^{\circ}\text{SH min}^{-1}$ can be used for approximating the incubation period. It is valid only for a narrow interval and may not be used in the exact mathematical description of the process.

1.9. Determination of the steady state of continuous fermentation

The curves of growth and product formation in the running-up period of fermentation were not determined, they were known from the batch kinetic measurements. The culture was checked only at a few points in order to obtain a picture of sound development.

The steady degree of acidity to be expected with a given dilution rate was graphically determined on the basis of the curves of product formation. (A line having a slope corresponding to the dilution rate was drawn from the point corresponding to the degree of acidity of fed-in milk. The abscissa of the intersection of this line with the rate curve of product formation provided the steady degree of acidity to be expected.) The value thus obtained showed a close correlation (within 1–2 °SH) with the values obtained experimentally.

Rendering the process continuous was started with each dilution rate below and above the pH value corresponding to the steady state to be expected, thus the steady state was approximated from both sides.

1.10. Sensory evaluation of the product

The products were judged organoleptically by scoring. A panel consisting of three members scored two samples each of the products obtained by the use of inocula of various dilution rates. Results were evaluated by analysis of variance.

2. Results

Continuous inoculum production was made at dilution rates 0.60, 1.20 and 1.50 h^{-1}, respectively. The pH values as formed at various dilution rates are illustrated in Figs. 2–4.

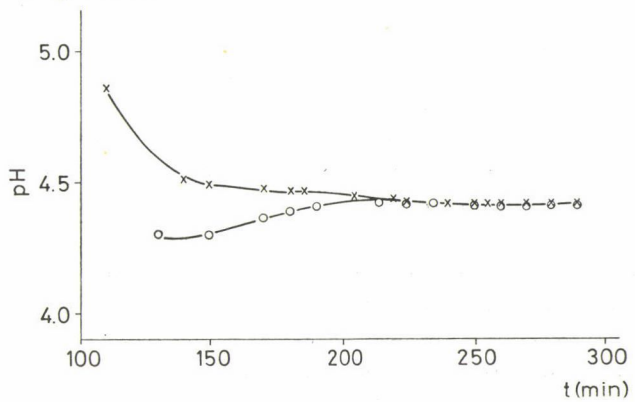

Fig. 2. Continuous yoghurt fermentation at a dilution rate of $D = 0.6\ h^{-1}$. Changes in pH till reaching steady state. —×—×— Third fermentation; —○—○— Fourth fermentation

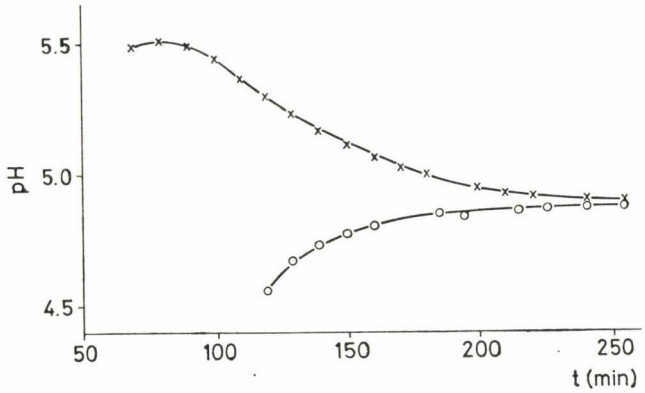

Fig. 3. Continuous yoghurt fermentation at a dilution rate of $D = 1.20\ h^{-1}$. Changes in pH till reaching steady state. —×—×— Fifth fermentation; —○—○— Sixth fermentation

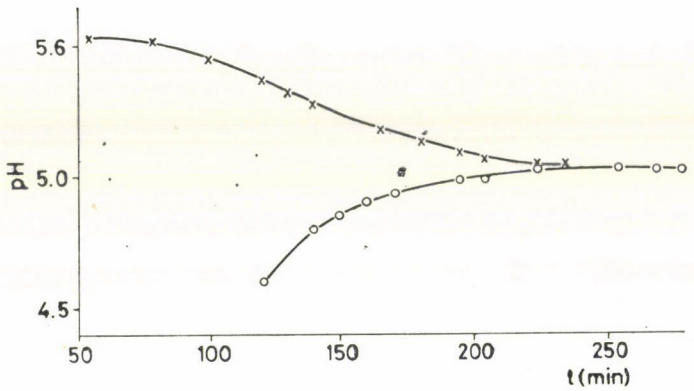

Fig. 4. Continuous yoghurt fermentation at a dilution rate of $D = 1.50\ h^{-1}$. Changes in pH till reaching steady state. —×—×— Seventh fermentation; —○—○— Eighth fermentation

The steady state was approximated from below and above at each inoculum prepared at different dilution rates. It can be seen in the Figures that approximation from both sides leads to identical results at identical dilution rates.

The main characteristics of yoghurts prepared with inocula obtained in batch and continuous fermentations are summarized in Table 1.

The ratio Str./Lb. shows the proportion of *Str. thermophilus* to *Lb. bulgaricus* in the inoculum. In the yoghurt prepared by batch fermentation with traditional clotting the proportion of cocci to rods met the required 1: 1, or at most 2: 1.

The sensory values obtained by scoring are given in Table 2.

Table 1

Parameters of steady state and the incubation time after inoculation required to reach 36 °SH, in continuous yoghurt fermentations

Fermentation	Dilution rate (h^{-1})	Steady state			Incubation period (min)
		(Str./Lb.)	pH	(°SH)	
1.	0.00	1:1	4.15	40.0	131
2.	0.00	1:1.2	4.20	39.0	128
3.	0.60	0.2:1	4.41	28.0	138
4.	0.60	1:1			135
5.	1.20	0.7:1	4.82	21.5	135
6.	1.20	0.4:1			137
7.	1.50	4:1	5.04	17.3	134
8.	1.50	0.4:1			138

Table 2

Sensory scores of yoghurt prepared with inoculum obtained in continuous fermentation

Panel member	Dilution rate of the continuous inoculum fermentation (h^{-1})				Control
	0.00	0.60	1.20	1.50	
1.	17.5	17.0	18.0	16.5	17.0
	17.5	17.5	17.0	17.0	17.5
2.	17.0	17.0	18.0	17.0	17.0
	17.5	17.0	18.0	17.5	17.0
3.	18.0	17.5	17.5	17.0	17.5
	17.5	17.5	17.0	17.0	18.0

3. Conclusions

The steady state of continuous fermentations was obtained mostly around the iso-electric point (4.7 pH) of casein, thus, precipitation of casein rendered counting rather uncertain. Only the Str./Lb. ratio could be established and that, too, inaccurately. In parallel fermentations the ratios are highly scattered, thus, their dependence on the rate could not be established.

Changes in the pH and in the degree of acidity are in complete concord with those theoretically expectable.

The incubation periods given in Table 1 were evaluated by analysis of variance. Results are shown in Table 3.

Table 3

Analysis of variance of incubation periods given in Table 1

Source of variance	SQ	DF	S^2	F	F_{95}
Total	86	7			
Between products	67	3	22.33	4.70	6.6
Between parallels	19	4	4.75		

No significant difference was found between the calculated F values and the critical ones, thus the use of continuous inocula does not extend the required incubation period.

Data obtained by analysis of variance of the scores in Table 2 are shown in Table 4.

Table 4

Analysis of variance of sensory scores given in Table 2

Source of variance	SQ	DF	S^2	F	F_{95}
Total	4.67	29			
Between products	3.17	14	0.23	2.30	2.5
Between parallels	1.50	15	0.10		

Comparing the calculated and tabulated F values the difference between yoghurts prepared with inocula of different dilution rates and those obtained on the market and used as the control, was not significant.

By rendering the fermentation of inoculum continuous, the fermenter volume as well as the incubation period may be substantially reduced. Instead of the incubation period of more than 150 min, in commercial production about 130–140 min are sufficient. Reduction is achieved by using identical milk of identical temperature for both continuous inoculum fermentation and the production of yoghurt and by the fact that the microflora of the inoculum is in the exponential growth phase. As a consequence of these conditions, the lag phase of growth in the final product is substantially reduced with consequent reduction of the incubation period while the quality of the product is equal to that of the commercial product.

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COMPARATIVE STUDIES ON THE SANITISING EFFECTS OF ETHYLENE OXIDE AND OF GAMMA RADIATION IN GROUND PAPRIKA

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Identical reduction of mesophilic aerobic cell count (2.5 orders of magnitude) was achieved by both treatments with ethylene oxide (600 g m^{-3}, 25 °C, 6 h) and a radiation dose of 5 kGy. With higher radiation doses, a more extensive reduction of cell count can be obtained.

In samples given the above radiation or ethylene oxide treatment neither coliform bacteria, nor *E. coli* I could be detected. Treatment with ethylene oxide caused no change in the mould count while a substantial reduction could be achieved by treatment with 5 kGy. The colour of ground paprika was practically not affected by either treatment.

Since an ionizing radiation dose of 5 kGy reduced the cell count to the same extent as treatment with ethylene oxide, the latter may be replaced satisfactorily by irradiation, simultaneously eliminating the problem of chemical residues.

Reduction of the cell count in seasonings is an important requirement of present-day food manufacture. Seasonings and, thus, also ground paprika used in the canning and meat industries, are contaminated by microorganisms. The high cell count in seasonings endangers the safety of canning and the shelf life of non-preserved meat products (BODROSSY, 1953). The microbial flora of seasonings is extremely varied. The high cell count in ground paprika is due mainly to growing and storage practices. Ground paprika may contain a total viable cell count of 10^4–10^6, a bacterial spore count of 10^3–10^4, an anaerobic cell count of 10^3–10^4, a coliform count of 10^1–10^3 and a mould count of 10^1–10^3 per g product (MURÁNYI *et al.*, 1971).

Aerobic and anaerobic spore-forming bacteria are a potential danger because they are present in the dormant state and therefore may survive processing and under unfavourable storage conditions may lead to quality degradation. The general demand for seasonings of reduced cell count increased in the Hungarian canning and meat industries, as well as in those of the importing countries. The introduction of methods for the reduction of cell count became necessary. First, reduction by heat treatment was suggested (BODROSSY, 1953). This, however, was not a final solution since the aroma and flavour substances in seasonings deteriorated upon heat treatment (LERKE & FARBER, 1960).

In consequence of the above problem cold sterilization methods came to the foreground. The biological activity of ethylene oxide was first observed

by COTTON and ROARK in 1928. The related research work was started by PHILLIPS and KAY during 1949.

CORETTI (1957) was the first to report on the viable cell count reducing (sanitising) effect of ethylene oxide in seasonings, this not accompanied by a reduction in their seasoning capacity. The above chemical (ETOX) is widely used for the sanitation of seasonings.

During treatment with ethylene oxide, toxic substances were found to develop (HILL, 1970). The effect of this treatment is not always satisfactory (MURÁNYI *et al.*, 1971). These circumstances indicate the search for a physical method satisfying more adequately the requirements.

Ionising radiations have been used for reducing cell count since 1948 (PROCTOR & GOLDBLITH, 1948). A radiation dose of 5 kGy ensures a reduction in the cell count and the destruction of pathogenic cells. Seasonings treated with 15 kGy may be considered practically sterile.

In Hungary TÖRÖK and FARKAS started cell reduction experiments by irradiation already in 1961. A treatment with 3–4 kGy reduced the cell count of ground paprika by 99.0–99.9% while it did not affect the soluble pigment content and the capsaicin content. FARKAS and co-workers found (1973) that ground paprika when treated with 4 kGy retained its microbiological stability even at high relative humidity.

In the present study, the efficiency of radiation treatment in comparison with ethylene oxide and changes in the colouring substances as a function of storage time, were investigated.

1. Materials and methods

1.1. Materials and packaging

Freshly ground paprika of "Delicatesse" quality was used in the experiments. In the industry, treatment with ethylene oxide is carried out in jute sacks holding 50 kg (Etox–I) or in polyethylene (90–100 μm) lined jute sacks (Etox–II). In the latter case the gas pervades the contents of the sack only through the narrow mouth. In order to save material the sacks were filled with wood shavings sterilized by irradiation and the ground paprika packaged in the traditional paper bags of 100 g capacity (red paper lined with parchment paper) were placed on the bottom, in the middle and on the top of the sack.

1.2. Treatments

Two different treatments were applied: ethylene oxide and gamma radiation. Untreated paprika was used as control.

1.2.1. Treatment with ethylene oxide. The treatment was carried out in *Degesh* gas chambers of 12.5 m^3 capacity at an ethylene oxide concentration of 600 gm^{-3}. The treatment lasted for 6 h at 25 °C. Composition of the gas: 10% CO_2 and 90% ethylene oxide.

1.2.2. Radiation treatment. Irradiation was carried out in the INSTITUTE OF ISOTOPES OF THE HUNGARIAN ACADEMY OF SCIENCES in a ^{60}Co panoramic radiation source of 2.1 PBq activity. The applied radiation doses were 5 ± 0.01, 9.3 ± 0.04 and 11 ± 0.03 kGy, respectively. Samples were placed in jute sacks lined with polyethylene.

1.3. Microbiological tests

The mesophilic aerobic cell count, spore number, coliform and *E. coli* I counts and mould count were determined by the most probable number (MPN) method in three parallels and 3 repetitions. The basic dilution was 10 g + 90 ml. Trypton—glucose—yeast (TGE), brillant green—gall—lactose—tryptophan and Sabouraud-maltose agar nutrient media were used. Tables contain logarithms of cell counts per g.

1.4. Solids content

This was determined according to HUNGARIAN STANDARD MSZ 9681–61 (1962).

1.5. Pigment content

The total pigment content expressed in capsanthin ($g\,kg^{-1}$) was determined according to BENEDEK (1958). The optical density of the solution was established with a *Bausch & Lomb* photometer at a wave length of 466 nm in a cuvette of 1 cm.

1.6. Mathematical evaluation

Results were evaluated by two-way analysis of variance (SVÁB, 1973).

2. Results

2.1. Sanitising effect of treatment with ethylene oxide and irradiation

Results of comparative analyses of the microbial count reducing effect of treatment with ethylene oxide and irradiation, resp., are shown in Fig. 1.

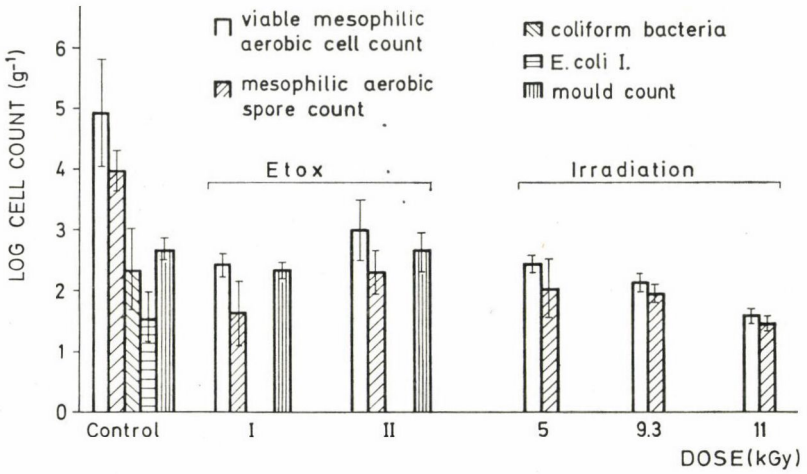

Fig. 1. Microbiological condition of ground paprika as a function of treatment with ethylene oxide (Etox-I – in jute sack; Etox–II – in jute sack lined with polyethylene membrane of 90–100 μm thickness) and of different radiation doses. The bars represent two standard deviations about the mean

It may be seen that the mesophilic aerobic count and the spore number in the samples packaged in two different ways and treated with ethylene oxide were reduced by nearly 2.5 log cycles. Results of analyses of variance are given in Table 1.

The evaluation of the effect of ethylene oxide on mesophilic cells and spores did not account for packaging. The calculated value of F was 285.55 as against 19.00 in the Table. This proves the efficiency of the treatment.

In Fig. 1 it is apparent, however, that the efficiency of treatment in the two differently packaged samples was different. In the jute sack lined with polyethylene (Etox–II) the cell count was reduced to a lesser degree, since the polyethylene membrane hinders the diffusion of the gas (VITÉZ, 1975). To check this an indicator sensitive to ethylene oxide was placed in various parts of the sack. On the basis of changes in colour it was established that the distribution of the gas was not uniform. Probably the lower sanitising effect is due to this fact.

Radiation treatment with 5 kGy gave an effect similar to the treatment with ethylene oxide. By increasing the dose to 9.3 kGy the reduction of cell count was of the order of 3, while at 11 kGy it was 3.5. The results of radiation treatment are shown in Table 2.

The calculated value of F was 982.79, *i.e.* very high as against the value of 9.28 in the Table.

Neither coliform bacteria, nor *E. coli* I could be detected in the samples treated with ethylene oxide or radiation. Probably these microbes are sensitive to both treatments.

Table 1

Analysis of variance of the effect of Etox treatment on the mesophilic aerobic cell count

Storage period (month)	Average log cell count		
	Packaging method		
	Untreated	Etox–I	Etox–II
1	5.06	2.36	3.68
3	5.00	2.75	3.30
4	5.13	2.30	3.18
5	5.38	2.69	3.07
6	4.80	2.40	2.50

Factor	Sum of squares	Degrees of freedom	s^2	$F_{calc.}$	$F_{5\%}$
Total	91.82				
Treatment	85.204	14	6.086	55.13	2.48
A (time)	0.78	4	0.195	1.773	6.39
B (Etox)	62.82	2	31.41	285.55	19.00
Effect of A×B	1.59	8	0.199	1.81	3.44
Error	6.62	60	0.11		

$t_{5\%} = 2.00$
$SD_{5\%} = \pm 0.651$

In relation to mould count, however, a substantial difference was observed between the two treatments. After treatment with ethylene oxide the mould count remained unchanged ($10^3\,g^{-1}$), while moulds were not detected in irradiated samples. The inefficiency of ethylene oxide treatment in this respect was supported by the results of analyses of variance as shown in Table 3.

The calculated value of $F = 2.105$ was substantially lower than the value of 19.00, found in the Table. This proves that treatment with ethylene oxide did not reduce mould count. The mesophilic aerobic cell count was determined in the untreated, ethylene oxide and radiation treated samples as a function of storage time (Fig. 2), too.

Contamination in the untreated samples was not reduced. As shown by the analyses of variance, no changes were observed in the samples treated with ethylene oxide (Table 1: $F = 1.773$; $F_{5\%} = 6.39$) or in those irradiated (Table 2: $F = 1.50$; $F_{5\%} = 6.39$). No change was detected in the mould count of samples treated with ethylene oxide (Table 3: $F = 2.34$; $F_{5\%} = 6.39$).

Table 2

Analysis of variance of the effect of radiation doses and storage time on the mesophilic aerobic cell count

Storage time (month)	Average log cell count			
	Radiation dose (kGy)			
	Untreated	5	9.3	10.7
1	5.06	2.69	2.24	1.41
3	5.00	2.42	1.94	1.51
4	5.13	2.42	1.97	1.60
5	5.38	2.51	2.04	1.61
6	4.80	2.36	2.24	1.64

Factor	Sum of squares	Degrees of freedom	s^2	$F_{calc.}$	$F_{5\%}$
Total	189.55				
Treatment	184.60	19	9.716	156.71	2.15
A (time)	0.37	4	0.093	1.50	6.39
B (dose)	182.8	3	60.933	982.79	9.28
Effect of A×B	1.42	12	0.188	3.03	2.69
Error	4.95	80	0.062		

$t_{5\%} = 1.99$
$SD_{5\%} = \pm 0.34$

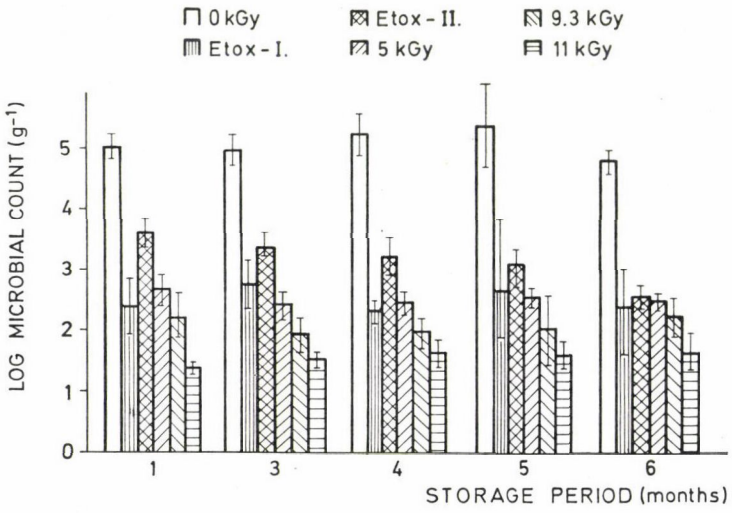

Fig. 2. Mesophilic aerobic cell count in ground paprika as a function of storage time after treatment with ethylene oxide (Etox–I – in jute sack; Etox–II – in jute sack lined with polyethylene of 90–100 μm thickness) or irradiation

Table 3

Analysis of variance of the effect of treatment by Etox and of storage time on the mould count

Storage period (month)	Average log cell count		
	Packaging method		
	Untreated	Etox–I	Etox–II
1	5.06	2.63	2.89
3	5.00	2.52	2.75
4	5.13	2.60	2.77
5	5.38	2.68	2.68
6	4.80	2.52	2.54

Factor	Sum of squares	Degrees of freedom	s^2	$F_{calc.}$	$F_{5\%}$
Total	35.73				
Treatment	7.029	14	0.502	1.073	2.48
A (time)	4.38	4	1.095	2.340	6.39
B (Etox)	1.97	2	0.985	2.105	19.00
Effect of A×B	0.66	8	0.083	0.177	3.44
Error	28.07	60	0.468		

$t_{5\%} = 2.0$
$SD_{5\%} = \pm 0.856$

2.2. *Changes in the pigment content*

The colour of ground paprika was studied as a function of storage time.

Figure 3 shows the changes upon treatment with ethylene oxide and Fig. 4 those upon irradiation.

As can be seen, the pigment content descreased as a function of storage time both in the control and in the treated samples. In order to establish whether the changes were affected by one or the other treatment, the results were studied by analysis of variance (Tables 4 and 5).

It is apparent that neither ethylene dioxide ($F = 0.507$) nor irradiation ($F = 6.371$) affected the pigment content. In both cases the effect of storage time dominated ($F = 36.993$ and $F = 195.429$).

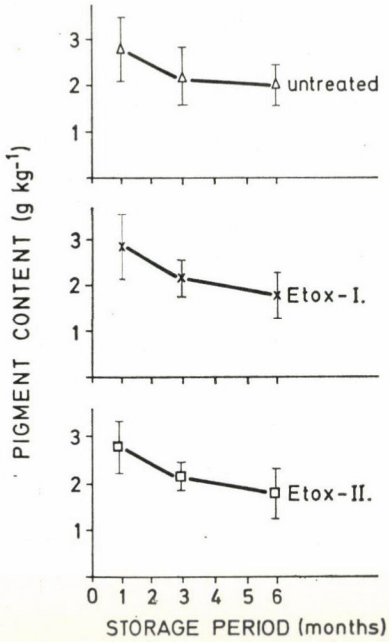

Fig. 3. Pigment content of ethylene oxide-treated ground paprika [according to BENEDEK (1958)] as a function of storage time

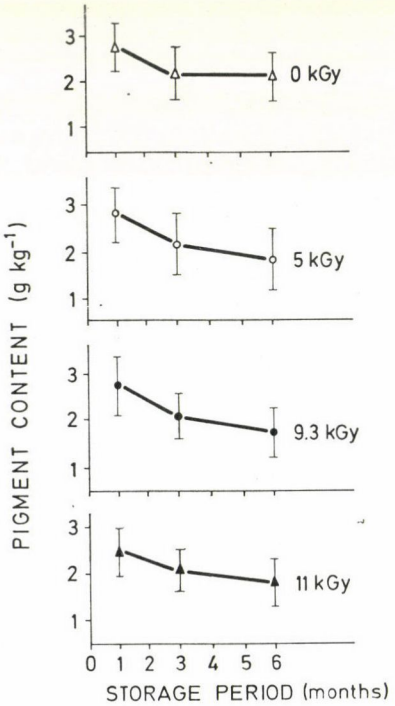

Fig. 4. Pigment content of irradiated ground paprika [according to BENEDEK (1958)] as a function of storage period

Table 4

Analysis of variance of the effect of treatment with ethylene oxide and of storage time on the pigment content

Storage period (month)	Average pigment content (capsanthin g kg^{-1})		
	Method of packaging		
	Untreated	Etox–I	Etox–II
1	2.75	2.75	2.75
3	2.26	2.25	2.29
6	2.02	2.75	1.73

Factor	Sum of squares	Degrees of freedom	s^2	$F_{calc.}$	$F_{5\%}$
Total	23.13				
Treatment	11.18	8	1.398	9.446	3.44
A (time)	10.95	2	5.475	36.993	19.00
B (Etox)	0.15	2	0.075	0.507	19.00
Effect of A×B	0.073	4	0.018	0.122	6.39
Error	11.95	81	0.148		

$t_{5\%} = 1.99$
$SD_{5\%} = \pm 0.323$

Table 5

Analysis of variance of the effect of radiation and of storage time on the pigment content

Storage period (month)	Average pigment content (capsanthin g kg^{-1})			
	Radiation doses (kGy)			
	Untreated	5	9.3	11
1	2.75	2.81	2.75	2.65
3	2.26	2.19	2.10	2.15
6	2.02	1.81	1.87	1.75

Factor	Sum of squares	Degrees of freedom	s^2	$F_{calc.}$	$F_{5\%}$
Total	18.27				
Treatment	14.54	11	1.322	37.771	2.82
A (time)	13.68	2	6.840	195.429	19.0
B (dose)	0.67	3	0.223	6.371	9.28
Effect of A×B	0.18	6	0.031	0.886	4.28
Error	3.73	108	0.035		

$t_{5\%} = 1.98$
$SD_{5\%} = \pm 0.504$

3. Conclusions

Seasonings and thus paprika, too, are used in their natural condition or as extracts. The latter mode of application is not always devoid of complications (chemical changes may occur) and the effect upon taste is not always identical with that of the seasonings used in their natural form. Microbial contamination of seasonings may cause problems in processing technology (products are not stable). Therefore different treatments are applied to reduce cell count.

Heat treatment is often not sufficiently effective and reduces flavouring capacity. Ethylene oxide and propylene oxide are frequently used in practice. Both reduce the cell count satisfactorily. However, ethylene oxide may form toxic compounds with other substances present (*e.g.* ethylene chlorohydrine) (WESLEY *et al.*, 1963; CORETTI & INAL, 1969; GOTTSCHALK, 1977; CORETTI, 1978). In the United States, the acceptable upper limit for ethylene chlorohydrin is 300 ppm. The ethylene chlorohydrin formed is a relatively stable compound and its quantity depends on many conditions (temperature, moisture content, chloride ions present, *etc.*). At values below the limit (*e.g.* 100 ppm) no deleterious effect was observed in animal experiments (BARNA, 1978; HIRSCHBERG & BARNA, 1978).

The advantage of radiation treatment lies in the fact that chemical residues may not be reckoned with and the specified irradiation technology ensures reproducible results. The condition of introducing this method into industry depends on proving its wholesomeness. In animal feeding experiments toxic effect of treatment with 15 kGy could not be demonstrated (BARNA, 1974; 1976). To establish mutagenic effect *in vivo* and *in vitro* experiments were carried out with heat treated, ethylene oxide treated and irradiated samples. Neither growth-inhibiting, nor mutagenic effect could be observed in *Salmonella typhimurium* strains (CENTRAL FOOD RESEARCH INSTITUTE, 1977). Radiation treated ground paprika caused no teratogenic effect (IMMUNOLOGICAL DEPARTMENT, BIOLOGICAL INSTITUTE, EÖTVÖS LORÁND UNIVERSITY OF SCIENCES, 1978). None of the chemical, microbiological, mutagenicity or teratogenicity experiments, carried out in recent years, indicated that the treatment of seasonings with radiation would be unwholesome (FARKAS, 1979). In Hungary, the new FOOD LAW, enacted 1 January, 1977, regulates production and marketing of irradiated foods (FARKAS, 1978).

In the present experiments the sanitising effect of ethylene oxide and radiation treatment in ground paprika were compared. Upon treatment with ethylene oxide, the cell count was reduced from $10^7\ g^{-1}$ to $10^4\ g^{-1}$ (HADLOK & INAL, 1973). Agreement with the results obtained in the present experiments is satisfactory (2.5 orders of magnitude).

Irradiation with 3–4 kGy caused a reduction of 2–3 orders of magnitude

in the cell count as found by TÖRÖK and FARKAS (1961) and FARKAS and coworkers (1973). Depending on the initial cell count sterility was achieved with doses of 15–20 kGy.

The above data support the observations made in the present experiments: the mesophilic aerobic cell count was reduced by 2.5 log orders of magnitude upon irradiation with 5 kGy. The bacterial spore count was reduced by ethylene oxide and irradiation to the same extent. Coliform bacteria seemed to be sensitive to both treatments, their presence need not be accounted for. The mould count was not reduced by treatment with ethylene oxide while irradiation with 5 kGy caused their complete destruction. Another advantage of radiation treatment lies in the capacity to increase heat sensitivity in both aerobic (KAN *et al.*, 1957; FARKAS & ROBERTS, 1976; STEGEMAN *et al.*, 1977) and anaerobic bacteria (MORGAN & REED, 1954).

In the pigment content of ground paprika, neither treatment with ethylene oxide, nor radiation treatment caused changes of any importance. In comparison with the untreated sample the pigment content was reduced by only 0.5–0.7% by both treatments. The pigment content, however, diminished, independently of treatment, during storage. The extent of change was mainly determined by storage conditions (moisture content, temperature, oxygen, light, packaging material — FARKAS *et al.*, 1973; KISS *et al.*, 1974; VAJDI & PEREIRA, 1973; BECZNER & FARKAS, 1974).

Industrialization of irradiation is strongly affected by factors of economy. In determining the costs of radiation treatment, the capacity of the plant and its degree of utilization are the decisive factors. A detailed study of this subject was carried out by BALÁZS-SPRINCZ (1977). The date of irradiation of agricultural and horticultural produce is determined by the time of ripening and harvesting. The remaining capacity of the irradiation plant may be converted to products which are more independent of season (FARKAS, 1975). Better utilization of capacity reduces the costs of treatment for all products, thus for ground paprika, too. In the study prepared earlier (KISS *et al.*, 1970) it was found that the treatment of ground paprika increased the production costs by about 1000 Ft per ton. Treatment with ethylene oxide costs about the same.

The results of the study have shown that the industrial treatment of ground paprika with ethylene oxide may be replaced by irradiation in every respect, indeed, from a certain point of view, it is even superior. Wholesomeness tests could not detect deleterious effects. Contaminations found in practice do not require doses higher than 5 kGy. This bears advantageously on irradiation costs as well.

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THERMAL ANALYSIS OF SPICES DECONTAMINATED BY IRRADIATION

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The cell-count-reducing effect of ionizing radiations is well known. To reduce microbiological contamination in the most frequently used spices, ground paprika, black pepper and a mixture of seven spices, a radiation dose of 1.5 Mrad (15 kGy) was applied.

The aim of this investigation was to find out whether this dose caused significant changes in the spices which could be detected by thermal analysis.

The results unambiguously show that the applied dose does not cause significant changes detectable by thermal analysis. This finding supports earlier experiences according to which no structural changes, disadvantageously influencing utilization of radiation treated spices, are caused by similar or smaller doses.

The applicability of gamma radiation to reduce microbial contamination in raw materials, semi-products and final products of the food industry has been extensively studied in the CENTRAL FOOD RESEARCH INSTITUTE, Budapest. In the course of these studies experiments were carried out first with ground paprika (TÖRÖK & FARKAS, 1961; FARKAS *et al.*, 1962), later with other seasonings and mixed seasonings (FARKAS & KISS, 1968; INCZE & FARKAS, 1968; FARKAS *et al.*, 1972; BARNA, 1973; BECZNER *et al.*, 1974; FARKAS *et al.*, 1973; FARKAS, 1973; BECZNER & FARKAS, 1974; BARNA, 1975; FARKAS, 1975; CENTRAL FOOD RESEARCH INSTITUTE, 1977). The aim of these studies was to discover changes in the sensory qualities and chemical changes causing sensory or sanitary deterioration (toxic, mutagenic or teratogenic effects) along with the cell-count-reducing effect of irradiation.

In the present study we attempted to elucidate any radiation-induced changes in the structure or in the mutual affinity of organic compounds constituting the spices. Since spices are considered rather complex systems, a method was sought which would be suitable for the examination of the mixed systems themselves and not only of their individual components. It was hoped to facilitate measuring directly consequences of radiation energy introduction not detectable by methods involving extraction. On the basis of the above considerations our choice fell on the method of dynamic thermal analysis.

1. Materials and methods

1.1. Seasonings

The experiments were carried out with a ground paprika product of the PAPRIKA PROCESSING PLANT, Szeged, called "Szemcsés édes" (Granular sweet), black pepper and a mixed seasoning, often used in the meat industry, and packaged at the COMPACK PACKAGING ENTERPRISE, Budapest. The mixed seasoning consisted of the following components: ground paprika 55%, ground black pepper 14%, coriander 9%, allspice 9%, marjoram 7%, cumin 4% and nutmeg 3% (w/v). In order to obtain identically granulated products, marjoram and nutmeg were ground manually and the other components on a grinding machine. All were screened on a sieve of 0.3 mm mesh and mixed in the proportion given above.

1.2. Radiation treatment

The spice samples were filled in test tubes and treated in the *RH-gamma 30* type ^{60}Co radiation source of the CENTRAL FOOD RESEARCH INSTITUTE. At the time of treatment the activity of the source was 0.275 PBq (peta becquerel) and the dose rate 3.37 kGy h^{-1}. Radiation treatment was carried out at room temperature (24 °C). A dose of 15 kGy (1.5 Mrad) was applied. The thermal analysis was carried out 3–7 days after treatment.

1.3. Dynamic thermal analysis

The studies were carried out with a MOM OD–2 type *Derivatograph* (HUNGARIAN OPTICAL WORKS, Budapest) at temperatures gradually increasing with time (PAULIK *et al.*, 1958). In the course of the analysis, the thermogravimetric (TG), the derivative thermogravimetric (DTG) and the differential thermoanalytical (DTA) curves, the latter being characteristic of the change in enthalpy, were simultaneously determined. The measurements were carried out in platinum crucibles under a temperature gradient of 3 °C min^{-1}. Each sample weighed 100 mg. Measurements were carried out up to 250 °C.

1.4. Method of evaluation

The results obtained were evaluated by one- and two-way analyses of variance, respectively (SVÁB, 1967).

2. Results

The TG, the DTG and the DTA curves were plotted against temperature (T). Curves belonging to one sample each of ground paprika, black pepper and mixed seasoning are shown in Figs. 1–3.

It can be seen in the Figures that the character of curves obtained by the thermogravimetric analysis of different seasonings is identical.

Weight loss as a function of temperature, in paprika treated with 15 kGy and untreated, is shown in Fig. 4, in black pepper in Fig. 5 and in the mixed seasoning in Fig. 6.

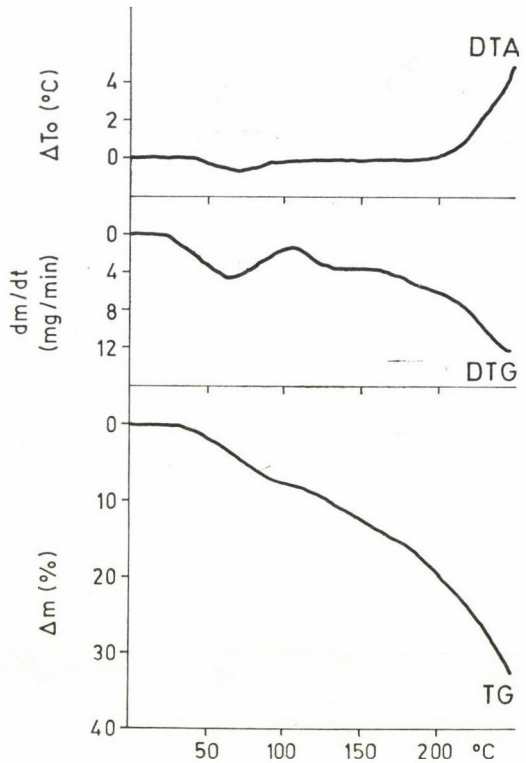

Fig. 1. TG, DTG and DTA curves of ground paprika

The results of analyses of variance of the weight loss data of treated and untreated ground paprika, ground pepper and mixed seasoning are given in Tables 1, 2 and 3.

As can be seen in the tables, the difference between results of parallel measurements is not significant (at the probability level of $P = 5\%$), thus, the average values belonging to the radiation treated and untreated samples are comparable.

Changes in weight belonging to the curves obtained by dynamic thermal analysis at temperatures of 50, 100, 125, 150, 175, 200, 225 and 250 °C, respec-

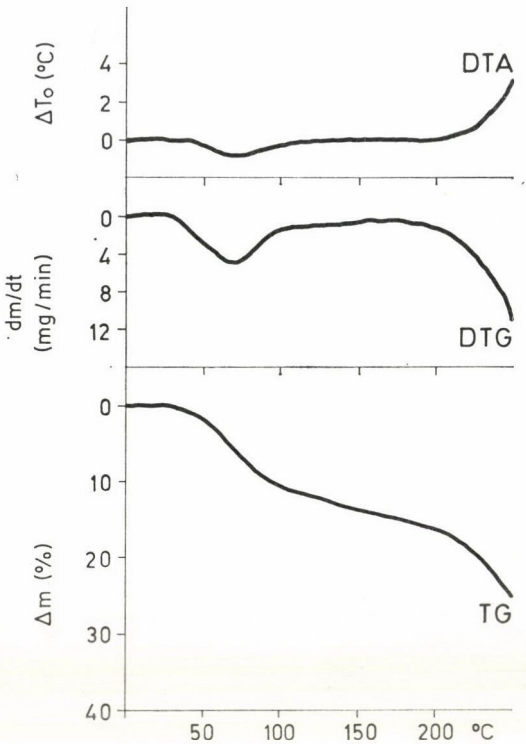

Fig. 2. TG, DTG and DTA curves of black pepper

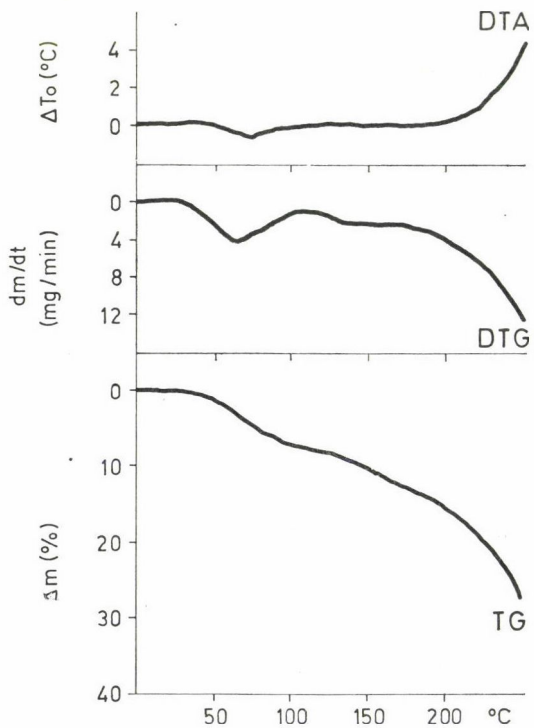

Fig. 3. TG, DTG and DTA curves of mixed seasoning

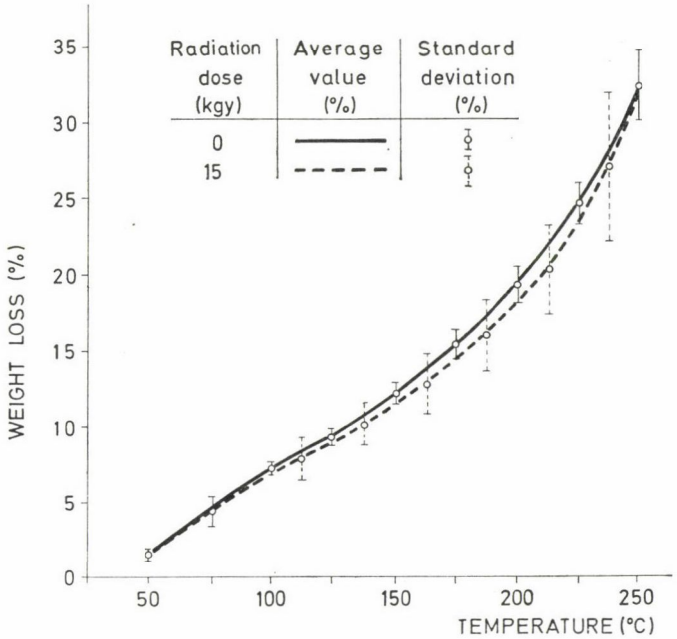

Fig. 4. The average of the thermogravimetric curves of 3 radiation treated and 3 untreated ground paprika samples and the standard deviations calculated for different temperatures

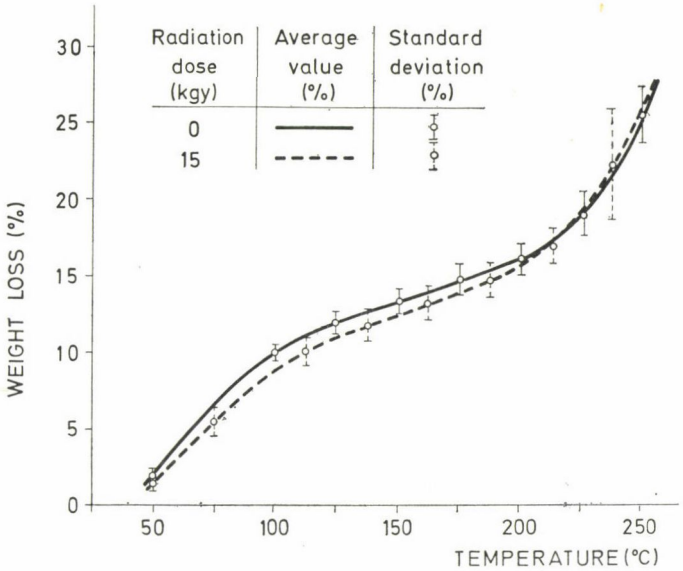

Fig. 5. The average of the thermogravimetric curves of 3 radiation treated and 3 untreated black pepper samples and the standard deviations for different temperatures

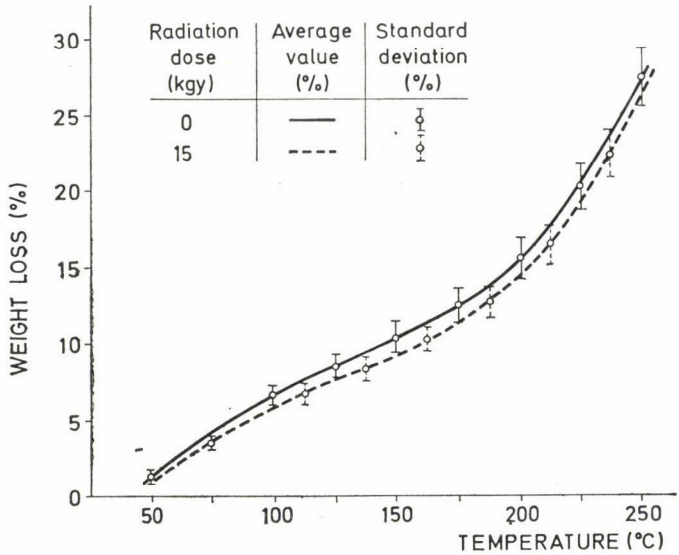

Fig. 6. The average of the thermogravimetric curves of 3 radiation treated and 3 untreated mixed seasoning samples and the standard deviations calculated for different temperatures

tively, were compared in order to establish whether the difference between changes in weight with temperature in treated and untreated samples is significant. The results of the two-way analyses of variance pertinent to ground paprika, pepper and mixed seasoning are given in Tables 4, 5 and 6.

The results unambiguously show that treatment with 15 kGy did not cause significant changes.

Table 1

Results of analyses of variance obtained by comparing weight loss data in radiation treated and untreated ground paprika as measured at different temperatures

Radiation dose	Deviations	Sum of squares	Degree of freedom	Variance	Calculated F value	F value from the table (P=5%)
0 kGy	Between parallel measurements	20.07	2	10.03	0.10	3.47
	Between weight losses as measured at different temperatures	2133.67	21	101.60		
	Between all measurements	2153.33	23	93.62		
15 kGy	Between parallel measurements	78.52	2	39.26	0.43	3.47
	Between weight losses as measured at different temperatures	1918.97	21	91.38		
	Between all measurements	1997.49	23	86.85		

Table 2

Results of analyses of variance obtained by comparing weight loss data in radiation treated and untreated ground black pepper as measured at different temperatures

Radiation dose	Deviations	Sum of squares	Degree of freedom	Variance	Calculated F value	F value from the table (P=5%)
0 kGy	Between parallel measurements	41.37	2	20.69	0.42	3.47
	Between weight losses as measured at different temperatures	1029.48	21	49.02		
	Between all measurements	1071.37	23	46.58		
15 kGy	Between parallel measurements	27.98	2	13.99	0.27	3.47
	Between weight losses as measured at different temperatures	1104.27	21	52.58		
	Between all measurements	1132.25	23			

Table 3

Results of analyses of variance obtained by comparing weight loss data in radiation treated and untreated mixed seasoning as measured at different temperatures

Radiation dose	Deviations	Sum of squares	Degree of freedom	Variance	Calculated F value	F value from the table (P=5%)
0 kGy	Between parallel measurements	7.04	2	3.52	0.05	3.47
	Between weight losses as measured at different temperatures	1388.64	21	66.13		
	Between all measurements	1395.68	23	60.68		
15 kGy	Between parallel measurements	5.65	2	2.82	0.04	3.47
	Between weight losses as measured at different temperatures	1335.47	21	63.59		
	Between all measurements	1341.12	23	58.31		

Table 4

Comparison of weight loss data in ground paprika to establish the effect of radiation treatment and temperature

Factors	Sum of squares	Degree of freedom	Variance	$F_{calculated}$	$F_{table\ value}$	
					P=5%	P=0.1%
Total	4160,45	47	88.52			
Treatments	4028.25	15	268.55			
Temperature	4014.16	7	573.45	138.85	2.32	5.12
Irradiation	9.67	1	9.63	2.33	4.15	13.29
Mutual effect	4044.53	7	572.08			
Error	132.21	32	4.13			

Table 5

Comparison of weight loss data in black pepper to establish the effect of radiation treatment and temperature

Factors	Sum of squares	Degree of freedom	Variance	$F_{calculated}$	$F_{table\ value}$	
					P=5%	P=0.1%
Total	2185.18	47	46.49			
Treatments	2116.50	15	141.10			
Temperature	2110.02	7	301.43	140.20	2.32	5.15
Irradiation	4.38	1	4.38	2.04	4.15	13.29
Mutual effect	2105.64	7	300.81			
Error	68.69	32	2.15			

Table 6

Comparison of weight loss data in mixed seasoning to establish the effect of radiation treatment and temperature

Factors	Sum of squares	Degree of freedom	Variance	$F_{calculated}$	$F_{table\ value}$	
					P=5%	P=0.1%
Total	9746.08	47	207.36			
Treatments	9682.60	15	645.51			
Temperature	2732.90	7	390.41	197.18	2.21	5.12
Irradiation	6.37	1	6.37	3.22	4.15	13.29
Mutual effect	2726.53	7	389.50			
Error	63.48	32	1.98			

3. Conclusions

Figures 4, 5 and 6, giving a summary of the study using dynamic thermal analysis to demonstrate the difference caused by radiation treatment prove these differences to be very slight. The two-way analysis of variance used to evaluate the differences (Tables 4, 5 and 6) proves that a treatment of 15 kGy has no significant effect. These findings support the results of dynamic thermal experiments carried out earlier (BECZNER *et al.*, 1974) in a higher temperature range. According to this experience, significant differences were not observed between treated and untreated paprika samples in the temperature range of 250–900 °C.

The practical consequence of these findings is that irradiation with 15 kGy, designed to reduce cell count in seasonings, does not cause changes detectable by dynamic thermal analysis.

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